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Phosphorus, Nitrogen, and Carbon Dynamics of Experimental Lake 303 during Recovery from Eutrophication[1]

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Levine, S. N., and D. W. Schindler. 1989. Phosphorus, nitrogen, and carbon dynamics of Experimental Lake 303 during recovery from eutrophication. Can. J. Fish. Aquat. Sci. 46: 2–10.

Little is known about the recovery of lakes from eutrophication, especially as it effects the cycling of nutrients other than phosphorus. We fertilized a naturally oligotrophic lake (Lake 303) in the Experimental Lakes Area, northwestern Ontario, with nitrogen and phosphorus over two summers and examined the subsequent recovery using mass balance and large in situ mesocosms. While large amounts of ammonium and smaller amounts of dissolved inorganic carbon (DIC) were released from the lake's aerobic sediments during the first year of recovery, phosphorus input from the sediments was not detectable during the same period. Chlorophyll *a* concentration closely tracked total phosphorus concentration, and both returned to prefertilization levels within 1 yr. In contrast, ammonium, nitrate, DIC, and dissolved organic carbon (DOC) needed almost 2 yr to reach baseline levels, and dissolved organic nitrogen (DON) even longer. The results show that different elements may recover at different rates and that the release of stored pools of nitrogen and carbon in the sediments plays a major role in delaying the recovery of these elements.

On connaît peu de choses sur le rétablissement des lacs suite à l'eutrophisation, notamment sur l'influence du phénomène sur le cycle des éléments nutritifs autres que le phosphore. Après avoir fertilisé un lac naturellement oligotrophe du nord-ouest de l'Ontario (le lac 303 dans la région des lacs expérimentaux) avec de l'azote et du phosphore pendant deux étés, nous avons examiné le phénomène de rétablissement subséquent à l'aide du bilan massique et de gros mésocosmes in situ. Bien que de grandes quantités d'ammonium et des quantités moindres de carbone inorganique dissous (CID) aient été libérées par les sédiments aérobies du lac pendant la première année de récupération, l'apport en phosphore des sédiments n'était pas détectable pendant cette même période. La concentration en chlorophylle *a* suivait de près la concentration de phosphore total et toutes deux sont revenues à leurs concentrations antérieures à la fertilisation en moins 1 an. Par contraste, il a fallu près de 2 an pour que l'ammonium, le nitrate, le CID et le carbone organique dissous (COD) reviennent à leur teneur naturelle et encore plus longtemps à l'azote organique dissous (AOD). Les résultats montrent que les différents éléments peuvent récupérer à des vitesses différentes et que la libération de l'azote et du carbone stockés dans les sédiments joue un rôle majeur dans le retard de la récupération de ces éléments.

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The eutrophication processes resulting from increased nutrient inputs are reasonably well understood today, having been studied extensively for more than 30 yr (Vollenweider 1968; Wetzel 1983; Henderson-Sellers and Markland 1987). Much less is known about the responses of nutrient cycles and biological communities as nutrient inputs are reduced. Most recovery studies have focused on the tracking of total phosphorus concentration, which usually diminishes rapidly when phosphorus inputs are curtailed. Phosphorus is removed from lakes both by hydraulic flushing and the settling of phosphorus-laden seston (Edmondson 1970, 1972, Edmondson and Lehman 1981; Michalski and Conroy 1973; Michalski et al. 1975; Schindler 1975). Occasionally, recovery is retarded by the return of phosphorus from sediments (Welch and Spyridakis 1972; Larsen et al. 1975; Ahlgren 1977).

The trajectory of total nitrogen during input reductions is poorly documented. Edmondson (1972) and Edmondson and Lehman (1981) reported on the nitrogen concentrations in Lake Washington after the diversion of municipal sewage away from the lake, and Schindler (1975, 1985) documented the response of nitrogen to stoppage of the experimental fertilization of Lake 304 with phosphate. However, neither of these experiments involved major changes in nitrogen inputs, and not surprisingly, total nitrogen concentrations were not substantially altered during the manipulation of either lake. For neither nitrogen nor phosphorus is much known about changes in speciation or cycling rates during lake recovery.

This paper assesses both nitrogen and phosphorus cycling during the recovery from eutrophication of Lake 303 in the Experimental Lakes Area, northwestern Ontario. This unstratified lake was experimentally fertilized with nitrogen and phosphorus over two summers and then allowed to recover. The

[1]Contribution No. ERC-124 of the Ecosystems Research Center, Cornell University.

paper also includes information on the response of carbon to the manipulations of nitrogen and phosphorus. The partitioning of recovery reactions among lake water, the atmosphere, and sediments was deduced from simultaneous observations made in large (78.5 m²) mesocosms placed near the mean depth of the lake and which were either open to or sealed from the sediments.

Lake Description

Lake 303 is a small (9.93 ha), shallow (mean depth = 1.5 m), headwater lake in the Experimental Lakes Area (ELA) of northwestern Ontario (49°39′N, 93°45′W). It is naturally oligotrophic, although less so than most of the unmanipulated lakes within the ELA (Schindler and Holmgren 1971). The terrestrial basin of the lake is 44.2 ha in area, composed almost entirely of rock ridges which are bare near their tops and covered with thin mats of mosses in lowland areas. The few pockets of soil (chiefly acidic podzols) are mostly less than 0.5 m deep. Sparse stands of jack pine (*Pinus banksiana*) are present along the ridge tops, while black spruce (*Picea mariana*) stands occupy the lower areas. The basin has never been logged or otherwise modified. The lake occupies a depression in the granite bedrock and thus is exempt from significant inputs and outputs of groundwater. It drains through a V-notch weir set in a rocky cleft at the south end of the lake. The water residence time of Lake 303 averaged 1.1 yr during the period of this study (Newbury and Beaty 1980).

Lake 303 is monomictic, with ice cover present usually from early November to early May. Its sediments are deeper than the water, flocculent, and highly organic (water content >90%, by weight; organic carbon content = 28% of dry weight (Brunskill et al. 1971)). Even before fertilization, the nitrogen, carbon, and phosphorus concentrations in its sediments were greater than those reported for most lakes (Brunskill et al. 1971) due to a very low contribution of clastic material to sediment bulk. As in other ELA lakes, the summer phytoplankton growth in Lake 303 is phosphorus limited (Sakamoto 1971; Healey and Hendzel 1980).

Materials and Methods

During the summers of 1975 and 1976, 3.02 g $N \cdot m^{-2}$ as sodium nitrate and 0.202 g $P \cdot m^{-2}$ as phosphoric acid were added to Lake 303. The fertilizer was split into 20 or 21 equal increments, with one increment added each week. Fertilizer was dissolved in 200 L of lake water and allowed to drip slowly into the lake during the week. Areal phosphorus loading was increased above background levels by about fourfold. Enough nitrogen was added to maintain a high N:P input ratio (the N:P ratio of the fertilizer was 15:1, by weight) and thus continue phosphorus-limited phytoplankton growth. Fertilization was discontinued in October 1976.

Samples were collected from the lake before (1970 and 1971), during (1975 and 1976), and after (1977 and 1978) manipulation. A biweekly sampling schedule was followed during the ice-free seasons of each year. In addition, samples were collected monthly during the winters of the premanipulation and fertilization periods. The premanipulation samples were taken at discrete depths (0, 0.5, 1.0, and 1.5 m) using a Van Dorn sampler or a peristaltic pump and tygon tubing, and the water was analyzed for a suite of chemicals, which included suspended phosphorus, nitrogen, and carbon, total dissolved phosphorus (TDP) and nitrogen (TDN), ammonium-nitrogen, nitrate-nitrogen, and chlorophyll *a*. The analytical procedures used were those of Stainton et al. (1974).

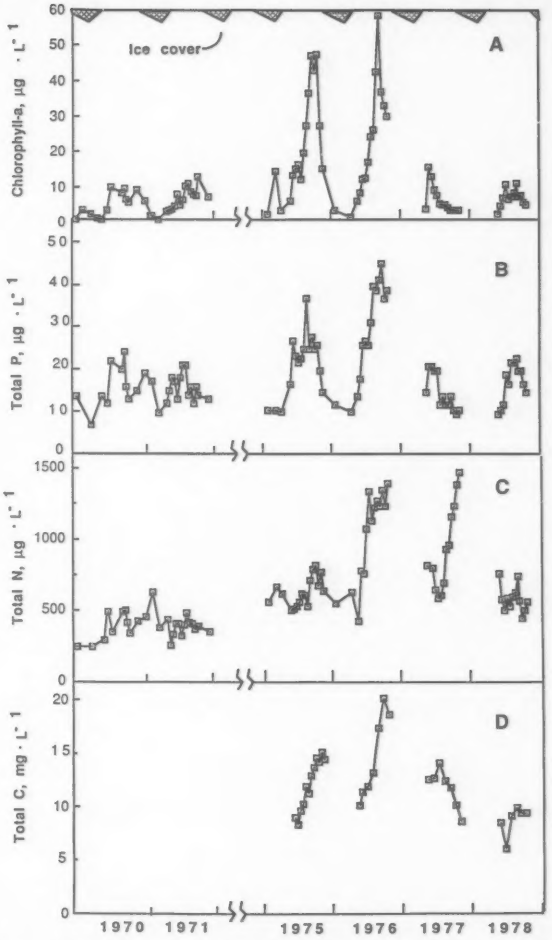

FIG. 1. Concentrations of (A) chlorophyll *a*, (B) total phosphorus, (C) nitrogen, and (D) total carbon in Lake 303 before (1970 and 1971), during (1975 and 1976), and after (1977 and 1978) experimental eutrophication.

During fertilization, we continued to take winter samples from discrete depths, but during the ice-free season, 2-m composite samples were taken. Samples were also collected from the lake's outflow at a V-notched weir which continuously monitored flow rates. In addition to the parameters measured in prefertilization years, analyses for this period included dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC), following Stainton et al. (1977). We calculated ambient dissolved CO_2 and the CO_2 concentrations expected from gas exchange with the atmosphere from DIC concentration, pH, and temperature using the equations and tables of Stumm and Morgan (1970). As CO_2 concentrations in ELA lakes vary dielly (Schindler and Fee 1973), the values that we report apply to midday.

We estimated the inputs and outputs of phosphorus, nitrogen, and carbon to the lake basin for the years of fertilization. Inputs included the nutrient in fertilizer, the quantity of which was

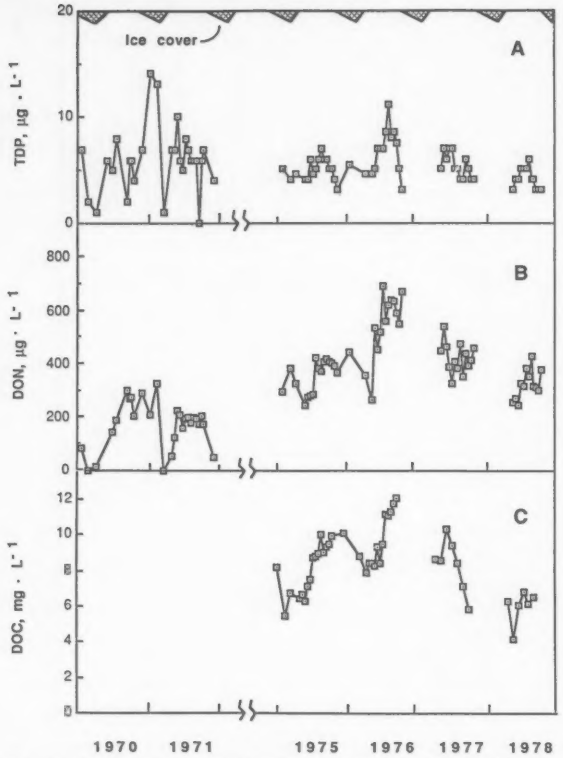

FIG. 2. Concentrations of (A) total dissolved phosphorus, (B) dissolved organic nitrogen, and (C) dissolved organic carbon in Lake 303 before (1970 and 1971), during (1975 and 1976), and after (1977 and 1978) experimental eutrophication.

precisely known, precipitation, and runoff. The amounts of nutrient entering in precipitation were calculated from the volumes and nutrient concentrations of rainfall collected daily at a nearby meteorological site (within 1 km of the lake) (Linsey et al. 1987). Nutrient inputs due to drainage basin runoff were estimated from data collected from the northwestern basin of Lake 239, which has soils and vegetation similar to those in the basin of Lake 303 and which has a stream outflow. We assumed that the rate of nutrient runoff per unit area from the two basins was similar and corrected the measurements of outputs from the 239NW basin for relative differences in the areas of the Lake 303 and 239NW basins. We calculated the loss of nutrient from Lake 303 via the outflow by multiplying the average nutrient concentration in the outflowing stream water by the discharge through the outflow weir during the period.

The lake monitoring program of the postmanipulation period was identical to that of the fertilization period, but was restricted to the ice-free season. In addition, between early August and late October of 1977, we conducted fertilization experiments in in situ mesocosms to assess the effects of N:P supply ratio on phytoplankton species composition and nutrient cycling (Levine 1983). The experiments included two unfertilized mesocosms, one of which was open to sediments at the bottom and the other fitted with a plastic floor, providing information on the role of sediments in the recovery of Lake 303. The mesocosms were cylinders of cross-woven polyethylene suspended from wooden–styrofoam frames at the lake surface (Cruikshank et al. 1983). They were 10 m in diameter and their weighted walls extended through 2 m of water depth and 0.5 m of sediments. We sampled the mesocosms (pooling three to five samples per mesocosm) on the same biweekly schedule as the lake and analyzed for the same suite of chemicals.

Results

Eutrophication

Fertilization resulted in a proliferation of phytoplankton and changes in phosphorus, nitrogen, and carbon cycling similar to those observed during earlier fertilization experiments at ELA (see Schindler 1975, 1985). In the initial weeks of fertilization, the chlorophyll *a* concentration in the lake roughly quadrupled (Fig. 1A). The summer phytoplankton community, which had been dominated by chrysophytes and diatoms in previous years, was now dominated by desmids and, to a lesser extent, cryptophytes (D. Findlay, pers. comm.; Levine 1983). Total phosphorus and total nitrogen concentrations also increased, but more gradually, reaching about 1.5 times background levels during the first summer of fertilization and three times background levels in the second (Fig. 1B and 1C). Because DIC and DOC were not measured before fertilization, the early effects of fertilization on total carbon concentration in Lake 303 are unknown. However, an increase in total carbon (DIC + DOC + suspended carbon) concentration between the first and second summers of fertilization (Fig. 1D) was observed, even though there was no artificial addition of carbon to the lake. In winter, when no fertilizer was added to the lake, total phosphorus and chlorophyll *a* concentrations diminished to prefertilization levels. By contrast, total nitrogen and total carbon declined but remained significantly above background concentrations, largely due to dissolved species (Fig. 2–4).

The phosphorus that accumulated in Lake 303 during fertilization was almost entirely sestonic. TDP concentration did not increase significantly (Fig. 2A; Table 1). In ELA lakes, TDP includes only miniscule amounts of phosphate (Levine and Schindler 1980; Levine et al. 1986) and is mostly dissolved organic phosphorus (DOP). That TDP did not change even though phytoplankton standing stock increased suggests that the phytoplankton in Lake 303 recycled most of the DOP produced through excretion and decomposition, a likely scenario given that the phytoplankton in the lake are phosphorus limited and produce phosphatases (Healey and Hendzel 1980).

Suspended nitrogen and carbon concentrations increased roughly in proportion to the increases in suspended phosphorus: all three suspended fractions were augmented by 1.5- to 2-fold during the first summer of fertilization and by 2- to 3-fold during the second (Fig. 3; Table 1). Like chlorophyll *a*, the suspended nutrient fractions returned to background concentrations in winter.

While phosphorus did not accumulate in dissolved organic compounds, carbon and nitrogen did. Dissolved organic nitrogen (DON, which is calculated by difference, TDN − (NO_3-N + NH_4-N)) concentration was 70% greater in 1975 and 270% greater in 1976 than the mean concentration in summers before fertilization (Fig. 2B; Table 1). In fact, about half of the nitrogen that accumulated in Lake 303 during fertilization was DON. DOC was not measured before 1975, but its concentration increased during the first summer of fertilization, from an average of 6.7 mg·L^{-1} between January and May to a mean concentration of 9.6 mg·L^{-1} in autumn. DOC

TABLE 1. Mean concentrations of major nutrient fractions and chlorophyll *a* in Lake 303 during the ice-free seasons of various years. Lake 303 was fertilized during the summers of 1975 and 1976. All concentrations are in $\mu g \cdot L^{-1}$. The sums of the means for the nutrient fractions sometimes do not equal the means for the concentrations of total nutrient due to occasional missing data.

Parameter	1970	1971	1975	1976	1977	1978
Chlorophyll *a*	5.8	7.2	24.4	24.9	5.5	6.1
Total phosphorus	16.2	14.9	22.1	30.0	12.8	15.3
Suspended phosphorus	10.4	8.6	17.1	23.2	7.5	11.3
Total dissolved phosphorus	5.3	6.2	5.0	6.8	5.3	4.1
Total nitrogen						
Suspended nitrogen	403	387	617	1082	992	563
Ammonium-nitrogen	165	167	243	432	149	171
Nitrate-nitrogen	11	19	5	46	290	48
Dissolved organic nitrogen	233	179	351	553	414	316
Total carbon	—	—	11 852	14 422	11 478	8 463
Suspended carbon	1399	1601	2 760	3 833	1 173	1 629
Dissolved inorganic carbon	—	—	597	1 250	2 013	998
Dissolved carbon dioxide	—	—	150	262	572	359
Dissolved organic carbon	—	—	8 202	9 663	8 186	5 860

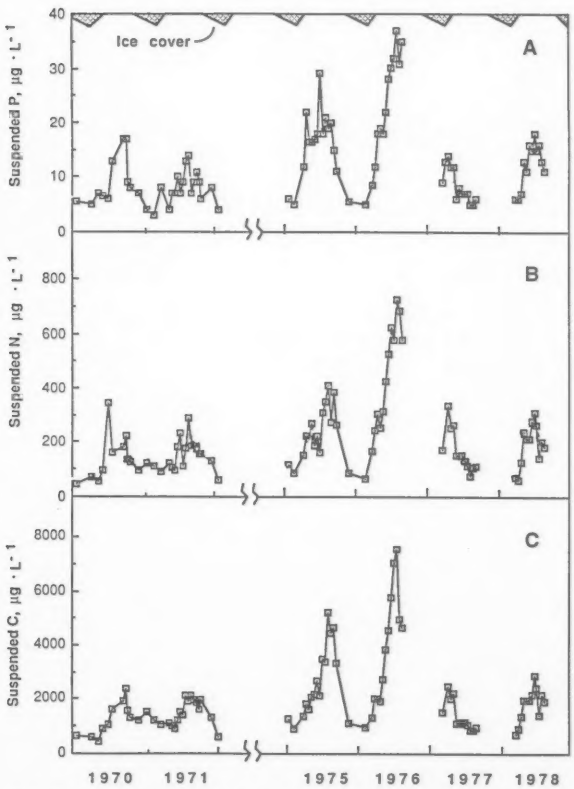

FIG. 3. Concentrations of (A) suspended phosphorus, (B) suspended nitrogen, and (C) suspended carbon in Lake 303 before (1970 and 1971), during (1975 and 1976), and after (1977 and 1978) experimental eutrophication.

concentration was an average of 17% greater during the second summer of fertilization than during the first (Fig. 2C; Table 1). Unlike chlorophyll *a* and the suspended nutrient fractions, neither DON nor DOC diminished in concentration in winter.

Although nitrate was the major component of our fertilizer, it usually did not accumulate during fertilization, but was rapidly incorporated into phytoplankton. The concentrations of both nitrate and ammonium varied with season. Ammonium, the main nitrogen product of decomposition and the form in which nitrogen usually diffuses from sediments, reached high concentrations (>100 $\mu g\ N \cdot L^{-1}$) when the lake was covered with ice (Fig. 4A). We believe that this accumulation reflected the low nutrient requirements of light-limited phytoplankton and severe oxygen depletion in the lower water column, which inhibited nitrification (Rheinheimer 1985). In spring, when algal growth resumed and oxygen became available to nitrifiers, the ammonium was rapidly depleted. Summer ammonium concentrations rarely exceeded 20 $\mu g\ N \cdot L^{-1}$ either before fertilization or during the first year of eutrophication. Nitrate concentrations were high only during spring overturn (Fig. 4B), when the ammonium that accumulated during winter was nitrified. Otherwise, nitrate concentration rarely exceeded 10 $\mu g \cdot L^{-1}$.

In the second summer of fertilization, the seasonal patterns for ammonium and nitrate concentrations showed some differences from previous years. Ammonium concentrations decreased in spring as usual but then began to creep up in value. They remained high (exceeding 200 $\mu g \cdot L^{-1}$ at times) until July. The ammonium peak did not coincide with increased nitrogen supply or with decreases in other nitrogen fractions; therefore, the ammonium probably came from sediments. In July, phytoplankton populations reached maximum densities (Fig. 1A) and presumably incorporated the excess ammonium, as ammonium concentrations quickly fell to within the normal summer range. A pulse of high nitrate concentration followed the summer peak in ammonium concentration (Fig. 4A and 4B).

DIC was not measured until the winter before lake fertilization. In ELA lakes, DIC undergoes seasonal cycles, reaching maximal concentrations in late winter, presumably because the CO_2 produced through respiration in winter is trapped under ice. In spring, the excess CO_2 escapes to the atmosphere. We found that DIC concentration was slightly higher during the second summer of whole-lake fertilization than during the first (Fig. 4C; Table 1). More interestingly, the gradient of CO_2 between the lake and the atmosphere differed

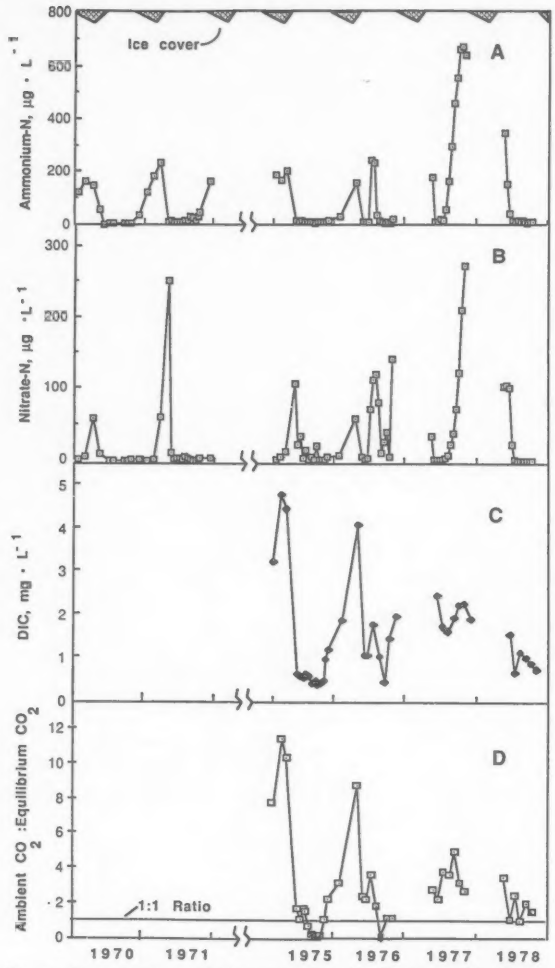

FIG. 4. Concentrations of (A) ammonium-N, (B) nitrate-N, (C) dissolved inorganic carbon, and (D) the ratio of ambient to equilibrium concentrations of CO_2 in Lake 303 before (1970 and 1971), during (1975 and 1976) and after (1977 and 1978) experimental eutrophication.

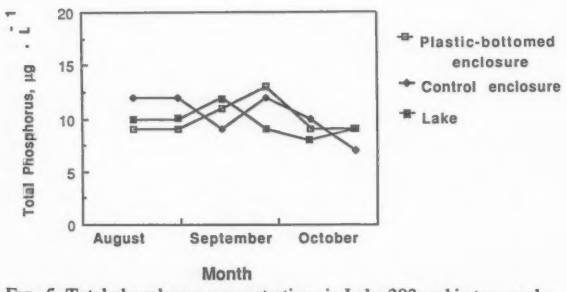

FIG. 5. Total phosphorus concentrations in Lake 303 and in two enclosures, one with and without floors, during August–October 1977.

during the two summers. During the summer of 1975, the gas was present at first at concentrations close to those expected at atmospheric equilibrium (Fig. 4D). As phytoplankton biomass

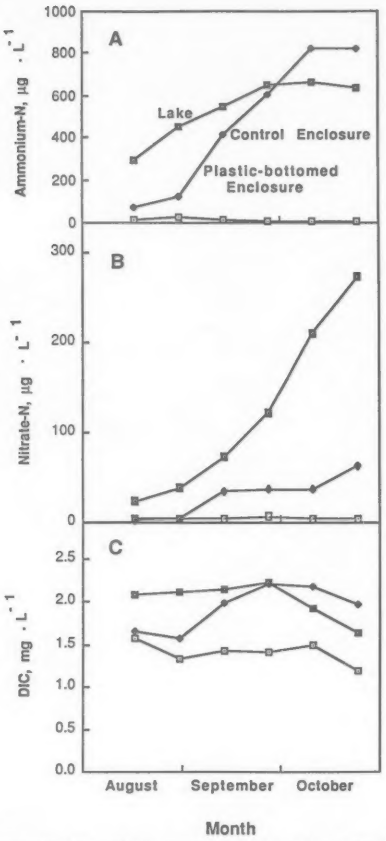

FIG. 6. Concentrations of (A) ammonium-N, (B) nitrate-N, and (C) dissolved inorganic carbon in Lake 303 and in the floorless and the floored enclosures during August–October 1977.

increased in response to whole-lake fertilization, however, CO_2 concentrations were drawn down to a small fraction of their equilibrium values. Apparently, at this time, photosynthetic use of CO_2 greatly exceeded respiratory production and a diffusion gradient for CO_2 was set up from the atmosphere to the water. By contrast, during the summer of 1976, CO_2 concentrations were below atmospheric equilibrium only for a short period, when phytoplankton biomass was at its maximum (Fig. 1A and 4D). At most times, CO_2 concentrations exceeded those that could be supported by atmospheric exchange alone. Thus, although chlorophyll *a* concentrations were similar during the two summers of fertilization, respiration must have been much greater during the second year. The period of high DIC and CO_2 concentrations was also the period when ammonium concentrations were elevated.

Recovery

In the spring following the termination of fertilization, chlorophyll *a* (Fig. 1A) and the suspended nutrient fractions (Fig. 2) increased in concentration as they had during the previous 2 yr when the lake was fertilized. We assume that phytoplankton were incorporating labile phosphorus that had remained in the water over winter. This spring "bloom" lasted only a few weeks. As phytoplankton sedimented, total phosphorus was

TABLE 2. Input of nitrogen (TDN + suspended N) to Lake 303 and the control mesocosm through atmospheric precipitation and terrestrial runoff between August 30 and October 11, 1977, versus the accumulation of ammonium-N and nitrate-N in the two systems during this period.

System	Surface input of nitrogen ($\mu g \cdot L^{-1}$)	Increase in ammonium-N ($\mu g \cdot L^{-1}$)	Increase in nitrate-N ($\mu g \cdot L^{-1}$)
Lake 303	133	215	173
Control mesocosm	10	700	32

reduced to concentrations below those measured before fertilization (Fig. 1B). Total phosphorus concentrations remained below or at the levels measured before whole-lake fertilization throughout the remainder of the postmanipulation period, suggesting that only insignificant amounts of phosphorus were released from sediments during Lake 303's recovery. Low phosphorus flux from sediments was verified by the results of the mesocosm experiments in 1977: both the lake and the open-bottomed mesocosm had the same mean total phosphorus concentration (10 $\mu g \cdot L^{-1}$) as the plastic-bottomed mesocosm, which was isolated from sediment inputs (Fig. 5).

As phosphorus sedimented from the water column of Lake 303 in spring 1977, chlorophyll *a* and other suspended nutrient concentrations plummeted. Throughout the remainder of our study, these parameters stayed well within the range of values observed prior to fertilization. Within the phytoplankton community, chrysophytes and diatoms regained dominance (Levine 1983).

DOC and especially DON (Fig. 2) responded to diminished nutrient supply much more slowly than chlorophyll *a* and the suspended nutrient fractions. During the summer of 1977, DOC was present at concentrations similar to those attained during the summer of 1976. In autumn, however, it returned to background concentrations. DON concentration remained above background throughout the 2 yr of postfertilization study.

The responses of DIN and DIC to reduced nitrogen and phosphorus inputs were complex. In the spring following the discontinuation of fertilization, first ammonium and then nitrate fell to low concentrations as ammonium was nitrified and as both ammonium and nitrate were incorporated into phytoplankton (Fig. 4). But in midsummer, the concentrations of the two species began to rise, just as they had in 1976. Unlike in 1976, little phytoplankton biomass was present in 1977 to use DIN. By autumn, ammonium concentrations exceeded 650 μg $N \cdot L^{-1}$, extremely high levels for an aerobic system, and nitrate concentrations peaked at about 270 μg $N \cdot L^{-1}$. Ammonium and nitrate also accumulated in the open-bottomed mesocosm (here, ammonium concentrations reached 820 μg $N \cdot L^{-1}$ and nitrate concentrations about 60 μg $N \cdot L^{-1}$). In contrast, ammonium concentration stayed below 20 μg $N \cdot L^{-1}$ and nitrate concentrations below 5 μg $N \cdot L^{-1}$ in the mesocosm with a plastic bottom (Fig. 6), indicating that sediments were the source of the nitrogen. The ammonium concentration in Lake 303 gradually diminished over the winter of 1977–78 and in the following summer reached background concentrations. Nitrate concentrations were also at background levels during the summer of 1978.

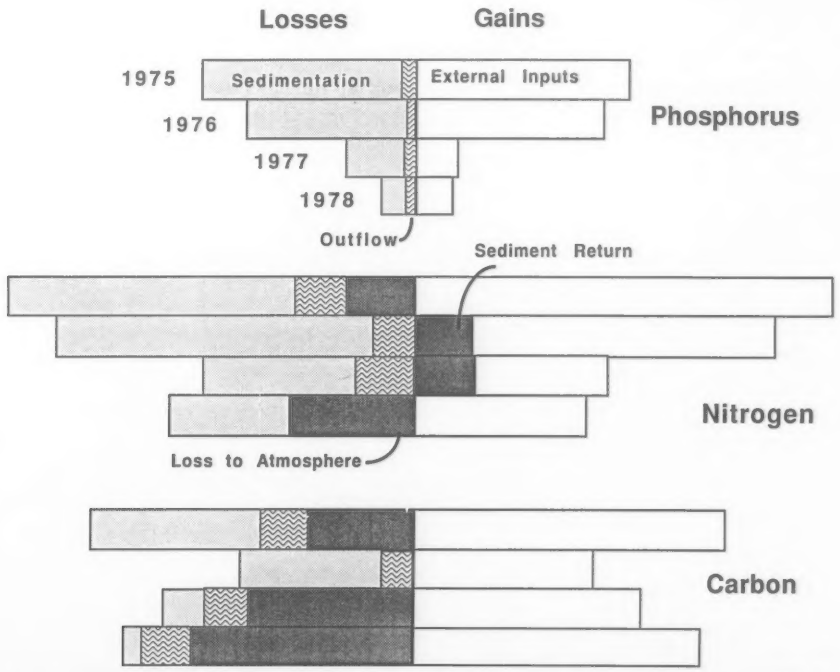

FIG. 7. Annual balances between phosphorus, nitrogen, and carbon inputs and outputs during 1975–78.

DIC concentration followed temporal trends similar to those for ammonium; DIC concentration increased sharply between July and October 1977, reaching concentrations much higher than those recorded during the summers of lake fertilization and then slowly decreased in 1978 (Fig. 4C). DIC concentrations were similar in the open-bottomed mesocosm (mean = 1.66 $mg \cdot L^{-1}$) and in the lake (mean = 1.68 $mg \cdot L^{-1}$). They averaged 30% lower in the mesocosm with a plastic bottom, suggesting that some DIC originated in sediments. CO_2 concentrations were greater than atmospheric equilibrium throughout the recovery period (in both the mesocosms and in the lake itself), but they were only about half as high in the lake in 1978 as in 1977 (Fig. 4D; Table 1). DIC did not represent a major portion of total carbon, however; even in 1977, it accounted for <20% of the carbon present.

Both total nitrogen and total carbon concentrations decreased when the fertilization of Lake 303 was discontinued (Fig. 1; Table 1). Total carbon concentration seemed to reach a new steady state by autumn 1977 whereas total nitrogen did not return to the range of 1970–71 values until autumn 1978.

Discussion

Our study of the water chemistry of Lake 303 during experimental manipulation of the supply rates of nitrogen and phosphorus uncovered both similarities and significant differences in the responses of the carbon, nitrogen, and phosphorus cycles to altered trophic state. During eutrophication, all three nutrients accumulated in seston, while dissolved inorganic nutrient pools remained largely at background levels. The cycles differed in that significant amounts of carbon and nitrogen were stored in dissolved organic compounds whereas the accumulation of DOP was undetectable. These results were largely as expected from other experimental studies of eutrophication within the ELA (Schindler 1975, 1985) and perhaps are characteristic of eutrophication responses in phosphorus-limited, oligotrophic systems.

When we stopped fertilizing Lake 303, phosphorus returned to background levels within months, while total carbon and nitrogen concentrations remained elevated for 1 and 2 yr, respectively. The phosphorus cycle was able to respond quickly to decreased inputs because phosphorus return from sediments was minimal (as indicated by the similarity of phosphorus concentrations in mesocosms with and without plastic floors) and because little phosphorus had been stored in slowly exchanging dissolved organic pools during eutrophication. Significantly, excess phosphorus was in seston and thus susceptible to sedimentation. Sedimentation must have been intense during the spring of 1977, for phosphorus returned to background levels much more quickly than the element could be flushed from the lake via flowthrough. In other ELA lakes, sedimentation velocities of 0.2–0.5 $m \cdot d^{-1}$ have been calculated (Hesslein et al. 1980; Levine et al. 1986; Bower et al. 1987), so that this would be expected.

Ammonium and nitrate accumulated in Lake 303 during the first summer of recovery from eutrophication. That the source of these nitrogen compounds was sediments is confirmed by mass balances for the mesocosms that were in the lake at this time (Table 2). In the untreated mesocosm open to sediments, much more nitrate and ammonium accumulated than could be accounted for by inputs at the surface (i.e. through precipitation). Nitrate concentrations were lower in this mesocosm than in the surrounding lake water, however, probably due to differences in nitrification rates and to the input of watershed nitrate to the lake but not to the mesocosm.

The negligible phosphorus feedback from the sediments of an ELA lake was not unexpected. Many radiotracer (Schindler et al. 1975; Levine et al. 1986), mass balance (Schindler et al. 1973, 1987), and porewater studies (Schindler et al. 1977, 1987) from other lakes in the ELA region have reached the same conclusion. However, these earlier studies have been concerned with the sediments of lakes fertilized for several years or unperturbed lakes. The current analysis was undertaken during reductions in external phosphorus inputs, a period most likely to yield a net flux towards the water. That no such flux occurred suggests to us that the desorption of phosphorus from sediments is a very slow process relative to sedimentation and adsorption or that much of the phosphorus released by decomposition in the sediments is immobilized by microbes (Doremus and Clesceri 1982).

We took advantage of the lack of phosphorus feedback from Lake 303's sediments to perform more discriminating mass balance calculations for phosphorus, nitrogen, and carbon than are possible when phosphorus sedimentation and phosphorus feedback are confounded. In addition to having no significant sediment source in Lake 303, phosphorus has no common natural gaseous forms (phosphine may exist, but has not been detected in sediments in measurable amounts; Stumm and Morgan 1970). Therefore, we calculated gross phosphorus sedimentation (S_P) from the formula $S_P = I_P - O_P - \Delta M_P$, where I_P is the total phosphorus input to the lake (the sum of the inputs from precipitation, runoff, and fertilization), O_P is phosphorus loss through the lake's outflow, and ΔM_P is the change in the mass of phosphorus in the water column over the period of interest (in our case, both 2-wk and annual intervals).

Gross nitrogen and carbon sedimentation (S_N and S_C) were calculated as the products of gross phosphorus sedimentation and the ratio of either nitrogen to phosphorus or carbon to phosphorus in seston: $S_N = S_P$ (suspended N : suspended P); $S_C = S_P$ (suspended C : suspended P). The above computation is accurate only if the C:N:P ratios in seston do not change during sedimentation. We were fairly confident that seston would not lose much nutrient in falling through Lake 303's very shallow water column (1.5 m deep, on average). Nevertheless, we used data from experiments performed in pelagic mesocosms in Lake 302S during 1978 (see Levine 1983) to test the hypothesis. In pelagic mesocosms, surface waters are isolated from sediments by the thermocline, which has a very low vertical diffusivity in small stratified lakes (Quay et al. 1980). Therefore, nitrogen sedimentation in these systems could be calculated in the same manner as phosphorus sedimentation, i.e. $S_N = I_N - O_N - \Delta M_N$ where I_N is the nitrogen input from the watershed, precipitation, and fertilizer, O_N is the N loss to outflow, and ΔM_N is the change in nitrogen mass in the water column. We compared suspended N : suspended P ratios in the mesocosms with the ratios of N sedimentation : P sedimentation. Because the epilimnion of Lake 302S was about 1 m deeper than the depth of Lake 303, differential sedimentation of nitrogen and phosphorus was likely to be greater in this lake than in Lake 303. For three of four of the test systems, the two variables were not found to be significantly different in magnitude; in the fourth, nitrogen was sedimented slightly more efficiently than phosphorus (Levine 1983).

Mass balance analysis for carbon and nitrogen yields a quantity for the exchange of an element between the water column and "internal" sources, which we will call F, equal to the sum

$I - O - S - \Delta M$. For carbon, F_C represents the sum of the carbon return from sediments and the net loss or gain of CO_2 across the atmosphere–water interface. It incorporates both daytime and nighttime CO_2 exchange at the water surface. For nitrogen, F_N is the net effect of nitrogen fixation, nitrogen return from sediments, and denitrification. Heterocystous blue-green algae are responsible for most nitrogen fixation in aerobic waters (Carr and Whitton 1982; Howarth et al. 1988), and phytoplankton samples collected routinely from Lake 303 during the years of chemical sampling indicated that heterocystous blue-green algae were exceedingly scarce (at their densest, these populations accounted for <5% of phytoplankton biomass). Therefore, we assumed that nitrogen fixation was negligible in Lake 303 and interpreted F_N as the difference between nitrogen return from sediments and denitrification. As is true for all terms calculated by difference, the error in F probably was large; F includes the residuals of the mass balance terms.

Our mass balance calculations indicated that during eutrophication, sedimentation was the major sink for carbon, nitrogen, and phosphorus alike (Fig. 7). But with the reduction of nitrogen and phosphorus loading in autumn 1976 and the decrease in phytoplankton biomass that followed, sedimentation rates diminished. Sedimentation continued to be the major phosphorus sink in the lake, but the importance of nitrogen and carbon sedimentation relative to atmospheric and outflow losses of these elements declined. All three nutrients sustained greater losses to sedimentation in 1977 than in 1978, possibly because in 1977 some fertilizer-derived nutrient remained in the water, available for biological uptake and sedimentation.

The values for F_N (the input of nitrogen from sediments minus denitrification) confirmed that nitrogen feedback from sediments was substantial during whole-lake fertilization: F_N = 0.775 $g \cdot m^{-2}$ in 1976 and 0.906 $g \cdot m^{-2}$ in 1977. F_N accounted for 14% of the total nitrogen input to Lake 303 in 1976 and for 34% of the lake's nitrogen input in 1977. Our estimates of F_N for 1975 and 1978, on the other hand, were clearly negative, suggesting that denitrification is normally substantial in this shallow lake. For example, in 1978, $F_N = -1.66$ $g \cdot m^{-2}$, a value similar in magnitude to the combined losses of nitrogen to other sinks. Thus, F_N is probably the difference between two large fluxes, both of which change in magnitude with shifting trophic state. Chances are that denitrification was actually greater in 1975 and 1976 than in 1978 because large amounts of nitrate, the substrate for denitrification, were added to the lake during these years, and denitrification is normally substrate limited in ELA lakes (Chan and Campbell 1980; Kelly et al. 1982). However, this nitrogen loss was obscured by the high rates of nitrogen return from sediments. The net return of nitrogen from sediments was greater in 1976 than in 1975, probably because sedimented material decomposes over a several month period and more decomposable material had accumulated in sediments by 1976 than by 1975. The high sediment return of nitrogen in 1977 suggests that material sedimented during eutrophication was still undergoing mineralization in the lake a year after fertilization was terminated.

Except during 1975, the first year of lake fertilization, large negative values were obtained for F_C (carbon input from sediments plus the net exchange of carbon with the atmosphere), indicating that in Lake 303, carbon exchange across the atmosphere–water interface is substantial and that the net flux is generally toward the atmosphere. This observation was substantiated by our calculations of midday CO_2 concentrations in the lake: except during the summer of 1975 and for a very short interval in 1976, this gas was present at concentrations above atmospheric equilibrium (Fig. 4D). At night, CO_2 concentrations are expected to be even greater than during the day because at this time, respiration occurs in the absence of photosynthesis. Thus it may be concluded that in Lake 303, decomposition normally exceeds primary productivity. This situation probably exists because the lake receives a significant burden of terrestrial litter, which decomposes in the water and sediments. That F_C was less negative during the summer of 1975 than during the recovery period is probably related to the elevated carbon demand of the phytoplankton during lake fertilization. In 1976, phytoplankton biomass, and thus CO_2 usage, was as great as in 1975. However, more phytoplankton-derived detritus had accumulated in the lake by then, and this detritus was undergoing decomposition. CO_2 production and the subsequent loss of this gas to the atmosphere decreases the value of F_C.

The magnitude of F_C is increased by the return of carbon from sediments as well as by CO_2 invasion from the atmosphere, and comparison of the DIC content of mesocosms with and without plastic floors (Fig. 6C) suggested that sediment feedback of carbon occurred during lake recovery from eutrophication. However, this source of carbon was masked by greater loss of carbon to the atmosphere. If we assume that during decomposition, carbon and nitrogen were released from detritus at the Redfield ratio, 6.6:1 by moles (Redfield 1958) or 5.7 by weight, we can obtain rough estimates of the carbon flux from sediments during lake recovery by multiplying F_N by 5.7. These estimates are unrealistically low, of course, since F_N includes denitrification. In fact, for 1978 we cannot estimate the carbon flux because F_N was negative during this period. For 1977, the calculation suggests that about 5 g of C was released per square metre of sediments. All of this carbon plus the 0.4 g $C \cdot m^{-2}$ that we estimated as F_C was lost to the atmosphere.

The slowness of the decline in DOC and especially DON concentrations in Lake 303 after the discontinuation of fertilization was probably due to low biological utilization of this carbon and nitrogen, as dissolved inorganic forms of these nutrients were relatively abundant and required less energy to acquire. Using a whole-lake addition of ^{14}C, Bower et al. (1987) found that the DOC in Lake 226 contributed little to whole-lake respiration, which agrees with our conclusion. To examine the relative importance of flushing and biological uptake as sinks for DON, we calculated the DON concentration that would be expected by autumn 1978 if DON were removed from Lake 303 by hydraulic flushing alone. We used the formula $C_t = C_a + (C_i - C_a)e^{-kt}$ where C_t is the expected concentration of DON in autumn 1978, C_i is the DON concentration at the time of fertilizer cutoff (late autumn 1977; 664 $\mu g \cdot L^{-1}$), C_a is the mean background DON concentration in summer (calculated for 1970 and 1971; 191 $\mu g \cdot L^{-1}$), k is the flushing rate in 1977 ($-0.64 \cdot yr^{-1}$), and t is time (1 yr). We found that the expected and actual DON concentrations were similar, 442 vs. 452 $\mu g \cdot L^{-1}$, confirming that biological uptake of DON was insignificant.

Summary

Phosphorus and algal biomass responded more quickly to the reduction of phosphorus and nitrogen inputs to Lake 303 than did nitrogen and carbon. Differences in the responses of the

cycles can be explained by the greater role of aquatic organisms in the cycling of phosphorus than in the cycling of carbon and nitrogen and by greater retention of phosphorus than of nitrogen and carbon by sediments (where again the retention may be due to greater biological immobilization). For Lake 303, gaseous exchanges were a major mechanism reducing carbon and nitrogen concentrations to steady-state conditions. Under all trophic conditions there was a net flux of carbon and nitrogen to the atmosphere (denitrification in the absence of nitrogen fixation). Neither element ever limited growth and both were probably expendable.

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Daytime Exposure to Fish Predation for Littoral Benthic Organisms in Unproductive Lakes

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Fish activity near the bottom was monitored in two central Ontario lakes using time-lapse video equipment. Predation risk for benthic invertebrates was measured as the frequency of fish entries into a hypothetical cylinder with dimensions such that an imaginary 2–3 mm invertebrate at the bottom-center of the cylinder would be within the detection radius of any fish entering the space. By this criterion, risk of predation is high, even in lakes with low fish densities. For 7 d of data spread over different lakes, sites, and months, the median rate of exposure of the hypothetical invertebrate to passing fish was 14.2 times/h; the median rate of exposure to fish that stopped or turned as if foraging was 3 times/h. Exposure to benthivorous fish varied markedly between two lakes in June and within a lake between June and August. Changes in the fish species, sizes, and level of activity all contributed to the variance. Exposure to fish also varied markedly among days, within a day, and among sites within a lake. Differences in predation risk between two neighboring sites were consistent for several days, suggesting that some of the patchiness in benthic invertebrate composition might be generated by patchy fish exploitation. The high median levels of exposure to fish suggest that benthic organisms should spend a high proportion of their time in risk-reducing positions or behaviors, so that competition among fish caused by resource depression will be important.

Nous avons surveillé les déplacements des poissons près du fond de deux lacs situés dans le centre de l'Ontario au moyen de matériel vidéo accéléré vue par vue. Les risques de prédation des invertébrés benthiques étaient fonction de la fréquence de passage des poissons dans un cylindre fictif, dont les dimensions étaient telles qu'un invertébré de 2-3 mm se trouvant au fond de la partie centrale du cylindre pouvait être repéré par tout poisson pénétrant dans cet espace. Avec cette méthode, les risques de prédation sont élevés, même dans les lacs où les densités de poisson sont faibles. D'après les données recueillies pendant 7 d dans divers lacs et sites durant des mois distincts, le taux médian d'exposition de l'invertébré fictif aux poissons de passage était de 14,2 fois/heure; le taux médian d'exposition aux poissons qui se sont arrêtés ou se déplaçaient comme s'ils se nourrissaient était de 3 fois/heure. L'exposition aux poissons se nourrissant d'organismes benthiques variait de façon marquée dans deux lacs distincts en juin et dans diverses zones d'un même lac de juin à août. Les changements observés quant aux espèces de poisson, leur taille et leur niveau d'activité ont tous contribué à cette variance. Les risques d'exposition aux poissons variaient également de façon notable d'un jour à l'autre, à divers moment d'une même journée et d'un site à l'autre dans un même lac. Les écarts observés quant aux risques de prédation dans deux sites voisins ont été constants pendant plusieurs jours, laissant entendre que la répartition irrégulière des invertébrés benthiques est peut-être due à l'exploitation non uniforme des poissons. Les niveaux médians élevés d'exposition aux poissons laissent supposer que les organismes benthiques devraient passer beaucoup de temps à se maintenir dans des positions ou à se comporter de manière à réduire les risques pour que la compétition entre les poissons augmente à cause de l'épuisement des ressources.

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On a small scale, laboratory studies of fish–benthos interactions have revealed a great deal about the behavioral, morphological, and chemical determinants of successful predation by fish, or successful defense by benthic organisms (e.g. Ivlev 1961; Ware 1973; Ringler 1979; Dill 1987). However, we know almost nothing about how often in the field a benthic invertebrate and a fish find themselves in the situations we create in the laboratory. How often is a benthic organism exposed to a foraging fish? What proportion of its time will be spent in a defensive behavioral mode? How does the frequency of exposures to fish vary through space and time? Are there relatively safe and more dangerous patches of benthic habitat for invertebrates; could there be large areas of benthic habitat that are poorly exploited by fish? Answers to these questions are necessary before we can correctly interpret the patterns we observe in larger scale field experiments and field surveys and before we can understand the selective forces that have molded fish foraging tactics and prey defenses.

In this paper, I describe an approach to answering these questions and present measurements of the overall level of exposure to fish predation in two unproductive shield lakes. I also present some preliminary data indicating how the exposure of benthic invertebrates to predatory fish can vary between lakes, among sites within a lake, and across seasons or within a day at one location.

Methods

Apparatus and Procedures

Direct observations of daytime fish activity over rectangular, 0.4–0.5 m^2 patches of littoral habitat were made with a time-

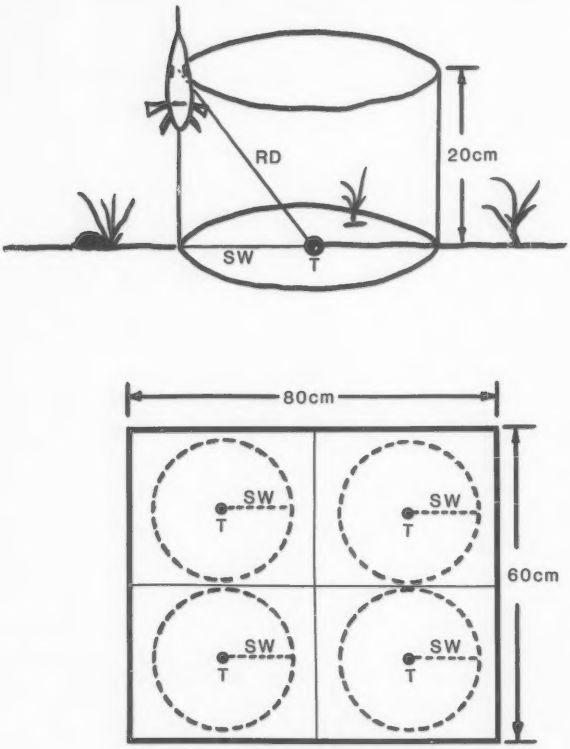

FIG. 1. Top: dimensions of hypothetical detection cylinders for imaginary invertebrate target (T), in relation to reactive distance (RD) and scanning width (SW) of a fish. Bottom: top view of four detection cylinders on the video screen during data transcription. Dimensions are real field dimensions. SW = 15 cm, corresponding to RD = 25 cm. The 20-cm height of the detection cylinders was estimated subjectively (see text).

lapse video recording system. A low-light, high-resolution black and white camera (Panasonic WV-1854) with a 12.5-mm auto-iris lens was placed in a plexiglass housing mounted on a simple stand made of galvanized pipe, which had been painted with a mottled, dark pattern to make it less conspicuous. The stand, with the camera attached about 1 m above the base and looking downward at 60–70° from horizontal, was lowered by rope from a small boat at depths of 1–4 m in the littoral zone. The nonvertical camera angle made it easier to distinguish shallow- and deep-bodied fish species. The housing was connected by 170 m of cable (Belden 9265) to a power source and a time-lapse video recorder (RCA TC3920) on shore. A high-resolution monitor (Panasonic WV-5410), whose underscan capability allowed the entire recorded video image to be viewed on the screen, was used to monitor camera performance and to transcribe data from tapes. Total cost of the system was about $7000 Canadian.

Clearly interpretable records of fish behavior were obtained when one image was recorded every 0.2 s, with playback at 60 frames/s. In the relatively clear water (Secchi depths > 4 m) of the subject lakes, natural light levels were adequate for recording from about 0.5 h before sunrise to 0.5 h after sunset. The scale of the image on the monitor was established by

TABLE 1. Mean and median rates of exposure of benthic organisms to fish per hour; all observation periods and sites pooled. Mean and median intervals between exposures (in minutes) are in parentheses.

	n[a]	Mean	Median	50% range
Fish entering detection volume	203	34.5 (1.7)	14.2 (4.2)	4.8–46.0
Foraging fish in detection volume	197	7.6 (7.9)	3.0 (20.0)	0.0–10.0
Fish feeding/m²	197	1.9	0.0[b]	0.0–0.0[b]

[a]Number of 0.5-h observation periods.
[b]163 observation periods included no feeding attempts.

lowering known-sized objects to several points in the monitored area just before the camera and stand were retrieved. The nonvertical camera angle meant that scaling factors differed slightly between the top and bottom of the screen. To simplify data transcription, these scaling factors were averaged and the camera was assumed to be looking vertically downward. A time–date generator built into the recorder allowed accurate time specifications.

To describe fish activity from the viewpoint of a benthic invertebrate prey item, I monitored the rate at which fish entered hypothetical, vertically oriented "detection cylinders" (Fig. 1, top) with a radius of 15 cm and a height of 20 cm from the bottom. A fish entering the upper rim of this volume could potentially detect a benthic prey item at the center of the cylinder bottom, if that prey could be detected at a radius ≥25 cm. This radius of detection is a conservative value selected from those measured for a variety of visually feeding centrarchid and salmonid fishes foraging for prey <3 mm in length (Ware 1971; Confer et al. 1978; Wright and O'Brien 1982; Henderson and Northcote 1985). Fish entering the detection volume in locations other than the upper rim could detect a prey at the bottom center with a detection radius <25 cm. Rate of entry by fish into detection volumes is therefore a conservative measure of exposure to predators from the point of view of a benthic invertebrate at the bottom center of the volume. One (for 1986 data) or four (for 1987) nonoverlapping detection volumes were delineated on the monitor screen for data transcription (Fig. 1, bottom). The upper boundary of the volume was estimated subjectively, but fish were usually obviously close to the bottom or clearly well above it, so that fine discrimination was not necessary. Rates of fish entry into the detection volumes were scored in 0.5-h blocks, one detection volume at a time, and the separate totals for each volume were averaged to produce an overall picture of fish activity in the approximately 0.5 m² viewed by the camera. With four volumes per screen, time required for data transcription was about one third of the original field recording time.

Fish activity in each 0.5-h block was expressed as the average rate of entry into a detection volume per hour. The subset of fish that stopped, turned, attacked an object, or mouthed the sediments within the detection volumes were designated foragers, on the assumption that they represented a more serious predation threat than those fish that were more clearly just passing through. Not all such designated foragers are necessarily motivated to feed if they detect a prey. However, unless a benthic invertebrate can immediately distinguish which ones are genuine threats, then from a prey's point of view they are all potential predators and should elicit the same defensive behaviors. Total entries by fish and entries by foragers into the detection volume are therefore both measures of predation risk

to a hypothetical organism located in the bottom-center of the cylinder. Actual feeding attempts, which involved attacks on objects or buccal contact with the bottom, were totaled for the whole visible area during each 0.5 h and expressed as an hourly rate per square metre.

Lakes

Video records were obtained over 3 d in August 1986 and 1 d in June 1987 in Gull Lake, Ontario (44°51′N, 78°47′W), and for 3 d in June 1987 in a shallow bay of Tondern Island, Lake Muskoka, Ontario (45°05′N, 79°30′W). Littoral fishes are feeding and growing rapidly from mid-May to mid-August in this region (Langford and Martin 1941; Keast 1978a). Littoral fishes in Gull Lake include pumpkinseed (*Lepomis gibbosus*), largemouth bass (*Micropterus salmoides*), smallmouth bass (*M. dolomieui*), rock bass (*Ambloplites rupestris*), yellow perch (*Perca flavescens*), Iowa darter (*Etheostoma exile*), white sucker (*Catostomus commersoni*), brown bullhead (*Ictalurus nebulosus*), and a variety of small cyprinids. Lake Muskoka supports a very similar list of species, with the addition of northern pike (*Esox lucius*) and walleye (*Stizostedion vitreum*).

Fish densities during video monitoring were estimated by comparing catches in large Windermere-like traps (Kushneriuk and Paloheimo 1984) with those from Lake Opinicon, Ontario, where I have used the same traps and where fish densities are well known. All of the fish species mentioned above are also present in Opinicon. Catches per trap-dy at the sites of the June 1987 recordings were 1.50 for Gull Lake (n = 16 trap nights) and 1.92 (n = 12) for Lake Muskoka. Values for Lake Opinicon were 2.72 fish/trap (n = 72) measured with the same gear at the same time of year and in similar depths and habitats. In Opinicon, fish biomass determined from seining is about 108 kg/ha (calculated from Keast 1965), and densities determined by strip counts in habitats similar to those I monitored with video are 0.69–0.98 fish/m² (Keast 1978a). If it is assumed that trap catches are proportional to both population density and biomass, fish densities for Gull Lake and Lake Muskoka are <0.6/m² and biomass densities are <75 kg/ha. Both these values are on the upper end of the range recorded for other shield lakes in Ontario (Chadwick 1976; Fraser 1981).

Results

Overall Exposure of Benthos to Fish

Pooling all observations across sites, seasons, and lakes (Table 1) shows that the level of potential exposure of benthic

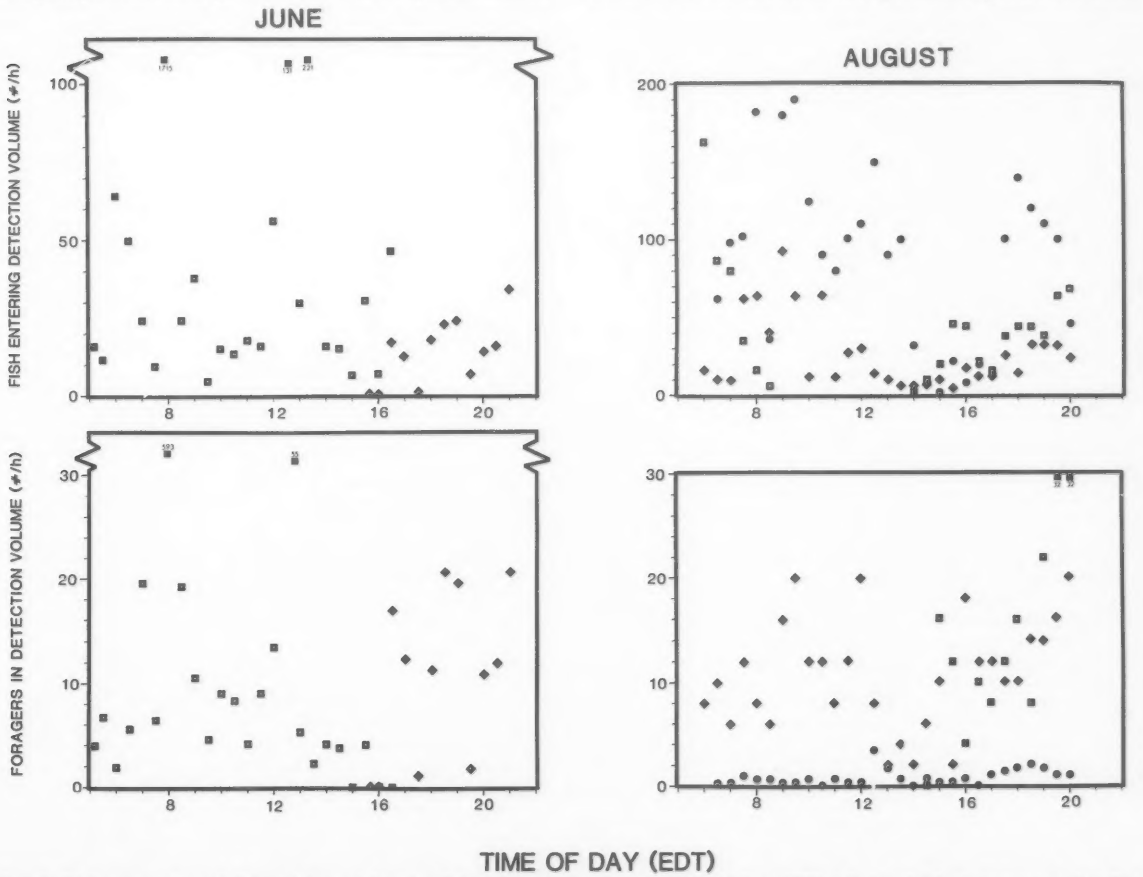

FIG. 2. Diel patterns in total fish activity and foraging fish activity during August 1986 and June 1987 at 2–4 m depth along a sandy shoreline with macrophyte cover in Gull Lake. Symbols represent different days and camera locations at this site. Note the different scales in the top panels. Feeding events were too rare to graph.

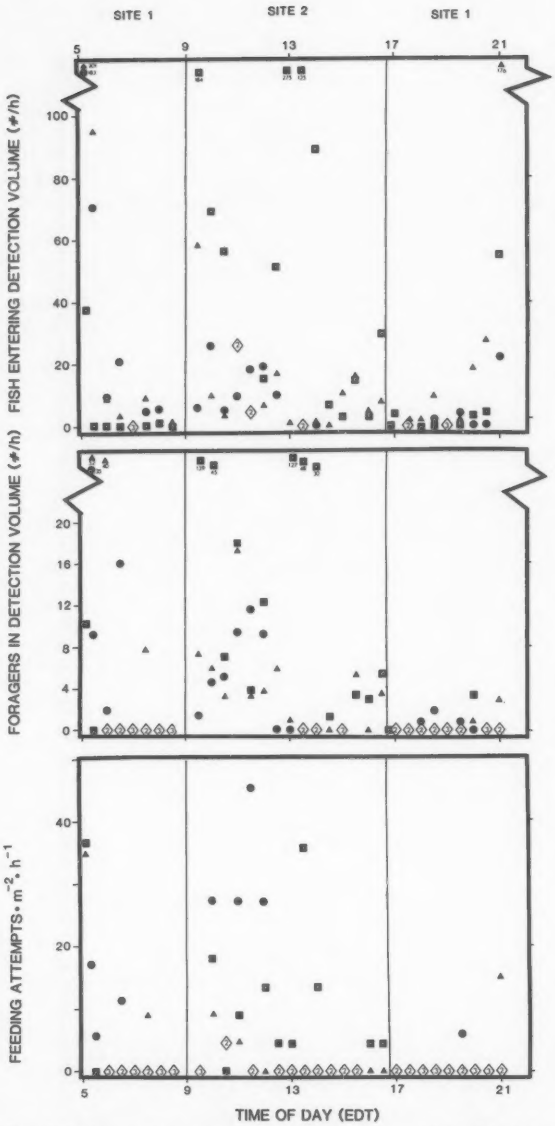

FIG. 3. Fish activity at two sites in a shallow bay of Lake Muskoka. Site 1 was 2.5 m deep with a sparse scattering of *Eleocharis* in fine sand and mud. Site 2, about 50 m from site 1, was 1.5 m deep with about 80 *Vallisneria* sp. and *Sagittaria cuneata* stems/m². Shapes of solid symbols indicate different days of observation. Numbers in open diamonds are the numbers of observations at the density indicated. Numbers next to symbols at the upper edge of each graph are ordinate values for outliers.

invertebrates to fish is very high. The median frequency that a benthic organism in a detection cylinder would be exposed to a passing fish is 14.2/h, or one exposure every 4.2 min. For foraging fish the median frequency is 3/h, or 20 min between exposures. The mean intervals between fish, 1.7 and 7.9 min for passing and foraging fish, respectively, are much shorter than the median intervals, indicating that the data are highly skewed by a few very intense bouts of fish activity.

Comparison among Months within a Lake

The fish in the June recordings from Gull Lake were <60 mm in total length, except for the occasional shoal of 2- or 3-yr-old perch; many of the small fish were probably largely planktivorous (Keast 1965). In contrast, fish <90 mm were rare at the same location in August, and most fish appeared to be 2- or 3-yr-old cyprinids, centrarchids, or perch, all of which are largely benthivorous (Keast 1965, 1977, 1978b).

The quantitative data (Fig. 2, top) suggest that total fish activity was higher in August than in June. However, a large portion of the fish observed in August were cyprinids or centrarchids in shoals that cruised through the field without turning or stopping. Therefore, while total activity in August was high, the rate of exposure to *foraging* fish was similar in both seasons. Given the size differences of the foraging fish in the two months, however, the threat of fish predation in August was certainly higher than that in June. These data are far too limited to suggest that this temporal pattern is a general one. They are presented to illustrate the complexity and variability of the fish predation threat in a single location.

Both total fish activity and foraging activity varied greatly among camera locations along a 100-m stretch of apparently homogeneous shoreline (Fig. 2, August), but because changes in both date and location are confounded in these data, I cannot determine which was responsible for the variation. The level of foraging at a site was not positively correlated with overall fish activity there.

Differences between Sites and Days

In Lake Muskoka the camera was moved approximately 50 m between an offshore and a nearshore site at the same times on three successive days in June (Fig. 3). In contrast with the small size of fishes active in Gull Lake only 7 d before, fish in Lake Muskoka were almost all pumpkinseed, rock bass, or yellow perch >100 mm total length. Feeding attempts were much more frequent than in Gull Lake, where only a few feeding events were observed.

The data (Fig. 3) reveal diel patterns of fish activity within a site and differences in activity among sites and among days within a site. Total, foraging, and feeding activity decreased rapidly after dawn (Fig. 3, site 1). An increase in total activity occurred at dusk in the same site, but foraging and feeding activity did not reach the high values characteristic of dawn. In Gull Lake (Fig. 2), there were no consistent activity peaks at either dawn or dusk.

Marked differences in all categories of activity occurred among days in both Lake Muskoka sites (Fig. 3). Ranges of rates for total, foraging, and feeding activity averaged over the 0900–1700 period in site 2 were 9–64/h, 4–32/h, and 1–13·m^{-2}·h^{-1} over the 3 d. The proportion of active fish that attempted to feed also varied among days; feeding rate was highest on the day that total activity was lowest.

Differences in fish activity between two sites were evaluated by comparing rates for the 2 h before and after site changes each day using the nonparametric Friedman's test with days as randomized blocks (Zar 1984). All three measures of fish activity were significantly higher for site 2 during the morning site change ($X^2 = 7.2$, df = 1, $p < 0.01$), and total and foraging activity were similarly higher for the afternoon change. Feeding rate for the latter change was almost nil for both sites.

Because site 1 was always monitored before site 2 in the morning and vice versa in the afternoon, the observed differ-

ence in activity between the two sites could be spurious if fish activity each day increased from 0700 to 1100 and decreased from 1500 to 1900. If anything, however, activity in site 1 decreased in the morning and increased in the evening (Fig. 3). It is also possible that the disturbance associated with introducing the camera may have temporarily altered fish behavior and affected the results. However, such a bias would influence site 2 in the morning and site 1 in the afternoon and would not be likely to produce the consistent difference seen in the data. The results are best interpreted, therefore, as real differences between the sites. Even though there are large variations among days, it appears that the threat of fish predation on benthic organisms can differ consistently between nearby locations.

Discussion

The results indicate that the median exposed littoral benthic invertebrate could be detected by 168 passing fishes, including 36 that stopped or changed direction as if foraging, in a 12-h day. In a site of high activity (Lake Muskoka's site 2), average intervals between exposures to foraging fish varied between 2 and 15 min over 3 d. These high levels of potential risk characterize lakes with littoral fish densities substantially lower than those commonly used in field studies of fish–benthos interactions. Fish biomass in my lakes was probably <75 kg/ha and density <0.6 fish/m^2 (see Methods). In comparison, Gilinsky (1984) used fish densities of 3.3–10 fish/m^2, Crowder and Cooper (1982) used a density of 1.3 fish/m^2 and a biomass of 240 kg/ha, Morin's (1984) fish density was 10.4 fish/m^2, and fish biomass in Boisclair and Leggett's (1985) study was 115 kg/ha.

The data indicate that risk of fish predation varies greatly among lakes, seasons, days, and sites within lakes. This high spatial and temporal variance would make it difficult to detect the effects of fish predation on benthic invertebrates and may be the major explanation for the lack of consistency in results of field experiments evaluating the impact of fish on the benthos (Thorpe 1986). Characterizing the patterns of fish predation risk in a lake with precision will require extensive sampling in time and space. Nevertheless, such characterizations are important prerequisites to our understanding the selective role of fish predation on the benthos.

The fact that differences in fish activity over two sites persisted for at least several days may be particularly important. It suggests that at least some of the well-known patchiness in the spatial distribution of benthic invertebrates may be related to patchy predation by benthivorous fish, and it leads to questions about what mechanisms produce high- and low-exploitation patches. Measurements such as those reported here, but more extensive, will tell us whether there are substantial areas of benthic habitat that are chronically underexploited by fish, perhaps because of the risk of piscivory.

The high frequency of exposures to fish in these shield lakes with relatively low fish densities suggests that invertebrate prey in most lakes will spend a large proportion of their time in postures and activity modes that minimize their risk of detection or capture by fish. Therefore, resource depression (Charnov et al. 1976), wherein fish reduce the food intake of other fish by changing prey behavior, without necessarily reducing prey density, should be an important form of competition within and among fish species.

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Effects of Oceanic Variations and the West Greenland Fishery on Age at Maturity of Icelandic West Coast Stocks of Atlantic Salmon (*Salmo salar*)[1]

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Investigations were conducted on the effects of oceanic variations (as measured by sea temperatures) and catches by the West Greenland salmon fishery on the sea age composition of Atlantic salmon (*Salmo salar*) stocks from 21 Icelandic west coast rivers. Annual ratios of grilse to two-sea-winter (2SW) salmon were strongly correlated among the 21 rivers. All eight rivers with time series extending back before the expansion of the West Greenland fishery showed lower ratios during the earlier period. Only 2 of the 21 rivers, however, had significantly declining ratios over their time series. In addition, for only one river was West Greenland catch significantly related to the ratios ($P < 0.05$), and for only one river did ratios increase when the expanded West Greenland fishery was active. Overall, the effects of the fishery on stock composition are evidently minimal. The mean April–May temperature when the smolts were to migrate out of rivers was significantly and positively related to subsequent ratios for five of the rivers, which, along with correlations among the ratios, indicated that more rapid growth of smolts in their first summer may have increased the ratios of grilse to 2SW salmon on several rivers.

On a étudié les effets des variations de la température de l'eau de mer et des captures des pêcheurs de saumon de l'ouest du Groenland sur la répartition par phases en eau salée des stocks de saumon de l'Atlantique (*Salmo salar*) dans 21 cours d'eau de la côte ouest d'Islande. Une étroite corrélation a été établie entre les rapports annuels castillons/saumons dibermarins dans les 21 cours d'eau. Dans les huit cours d'eau pour lesquels on disposait d'une série chronologique remontant à une période antérieure à l'intensification de la pêche dans l'ouest du Groenland, les rapports étaient initialement inférieurs. Cependant, d'après les données recueillies au fil des ans, les rapports n'ont diminué de façon significative que dans 2 cours d'eau parmi les 21 examinés au total. En outre, une relation significative entre les captures dans l'ouest du Groenland et les rapports (*P* inférieur à 0,05) n'a été observée que dans l'un des cours d'eau, et les rapports n'ont augmenté que dans une seule rivière lorsqu'il y a eu intensification de la pêche dans l'ouest du Groenland. Dans l'ensemble, les effets des captures sur la composition des stocks sont manifestement minimaux. La température moyenne en avril–mai, au moment où les smolts devaient quitter les cours d'eau, était reliée de façon significative et positive aux rapports ultérieurs dans cinq des cours d'eau, ce qui, de pair avec les corrélations établies entre les rapports, révèle qu'une croissance plus rapide des smolts au cours de leur premier été avait peut-être augmenté les rapports grilses/saumons dibermarins dans plusieurs cours d'eau.

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Recent investigators have sought to identify genetic and environmental factors affecting the age at sexual maturity of Atlantic salmon (*Salmo salar*) (Saunders 1986; see comprehensive reviews by Gardner 1976 and Meerburg 1986). Sea-age at maturity has been shown to have a genetic basis, and differences in mean sea-age *among* stocks have been correlated with large-scale environmental factors (Dahl 1916; Scarnecchia 1983). In addition, the environmental and developmental history of an individual fish has been shown to affect its ultimate sea-age at maturity (Thorpe 1986).

Although it is known that a complex of genetic and environmental factors influences sea-age at maturity, biologists have a poorer understanding of how specific genetic and environmental factors interact and influence age at maturity (Dempson et al. 1986). Investigators have found significant correlations between the number of grilse (i.e. having spent 1 yr at sea) in a stock in 1 yr and the number of two-sea-winter (2WS) salmon the next year (Kristjánsson 1982; Scarnecchia 1984b). There is also some evidence that sea-age at maturity *within* a year-class

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TABLE 1. Catch (in metric tons) of Atlantic salmon off West Greenland, 1960–85. Catch before 1960 assumed to be 0 for all analyses.

Year	Catch
1960	60
1961	127
1962	244
1963	466
1964	1539
1965	861
1966	1370
1967	1601
1968	1127
1969	2210
1970	2146
1971	2689
1972	2113
1973	2341
1974	1917
1975	2030
1976	1175
1977	1420
1978	984
1979	1395
1980	1194
1981	1246
1982	1077
1983	310
1984	297
1985	851

within a stock either is largely determined genetically or is influenced by environment mainly when the salmon are in freshwater (Chadwick et al. 1986).

However, some investigators of Atlantic and Pacific salmons have concluded that marine factors also influence annual variations in age at maturity. Peterman (1985) found that mean age at maturity varied similarly among Bristol Bay stocks of sockeye salmon (*Oncorhynchus nerka*) for fish of the same smolt class. He concluded that most of the variations in age at maturity probably resulted from conditions during the early marine life of the fish. Martin and Mitchell (1985) found that lower sea temperatures led to higher numbers of grilse relative to multi-sea-winter (MSW) salmon returning to the River Dee, Scotland. In contrast, Dempson et al. (1986) found no evidence of sea temperatures affecting sea-age at maturity of Atlantic salmon in the Restigouche River, Quebec, and other Canadian rivers.

It is not clear if or how oceanic conditions exert an effect on sea-age at maturity of Atlantic salmon. In addition, available data on age composition of salmon from rivers are often biased and confounded by age-selective fishing mortality from mixed-stock fisheries off the west coast of Greenland, in the Norwegian Sea, near the Faroe Islands, as well as from nearshore commercial fisheries.

Inasmuch as Iceland's west coast rivers are geographically close to the location of the West Greenland fishery and the total annual Icelandic harvest of wild salmon is only 200 tonnes (less than 8% of West Greenland catches by weight in the 1970s), it is reasonable to ask if the West Greenland fishery is harvesting large numbers of Icelandic salmon. Because oceanic fisheries off West Greenland harvest potential MSW salmon but not grilse (International Council for the Exploration of the Sea 1986), excessive offshore harvest of potential MSW salmon would be expected to lower mean age at maturity of fish returning to the rivers. Major reductions in age at maturity in these stocks might also be a useful indicator of overharvest (e.g. Paloheimo and Elson 1974) and potential loss of genetic diversity. Such harvests would, in turn, complicate attempts to assess environmental factors affecting sea-age at maturity.

In this paper, we investigate the effects of the West Greenland fishery and between-year variations in oceanic conditions on sea-age of the salmon stocks from 21 Icelandic west coast rivers. We seek to answer if and how age composition of the stocks (expressed as ratios of grilse to 2SW salmon) has changed coincident with fishing off West Greenland. We also investigate how interannual variations in oceanic temperatures affect the grilse to 2SW salmon ratios.

West Greenland Fishery

The West Greenland fishery began more than 200 yr ago, but catches were modest until the early 1960s when catches from a directed gillnet fishery rose rapidly from 60 tonnes in 1960 to more than 1500 tonnes in 1964 (Shearer and Balmain 1967; Pyefinch 1969; International Council for the Exploration of the Sea 1981; Jensen 1988). In 1965, an additional drift gillnet fishery at Greenland was begun by Scandinavians, which resulted in a total annual catch of over 2600 tonnes by 1971. Catches remained about 2000 tonnes per year until 1976, when a quota of 1190 tonnes was established. Catches from 1976 to 1984 were at or near established quotas (i.e. quotas were between 1190 and 1265 tonnes, and catches were between 984 and 1420 tonnes), except for 1983 and 1984 when unusually low oceanic temperatures (Stein and Buch 1985; Reddin and Shearer 1987) resulted in reduced availability of salmon, and catches dropped to 310 and 297 tonnes, respectively. Catches for 1985 were at quota levels of 850 tonnes (Table 1). Estimates of effort are not available (International Council for the Exploration of the Sea 1986). Fish caught are all potential MSW salmon (International Council for the Exploration of the Sea 1986). North American and European stocks of salmon contribute in similar or equal proportions to the fishery in most years (International Council for the Exploration of the Sea 1986; Reddin and Short 1986), and sex ratios are as high as 4.1:1 in favor of females (Pyefinch 1969; Jensen 1988), which tend to mature later and migrate farther than males in most salmon stocks.

Although tagged Icelandic salmon have occasionally been caught in the West Greenland fishery (Ísaksson 1980; Jensen 1988), few Icelandic stocks are tagged, and the tags recovered have been so few that it is not known how many Icelandic salmon are being harvested there.

Materials and Methods

Sea-age composition of Icelandic salmon stocks was determined by the use of long-term angling catch records from all 21 west coast rivers having significant percentages of MSW salmon (Fig. 1). Most of these rivers supported rod angling only. Detailed catch records, including number, weight, and sex of individual salmon caught, were available for recreational fisheries. However, significant commercial net fisheries are active in the Hvítá (Borgafjördur) system and in the Ölfusá–Brúará–Hvítá (OBH) system. A small net fishery was also active on Álftá. Data from these fisheries were poor, and the

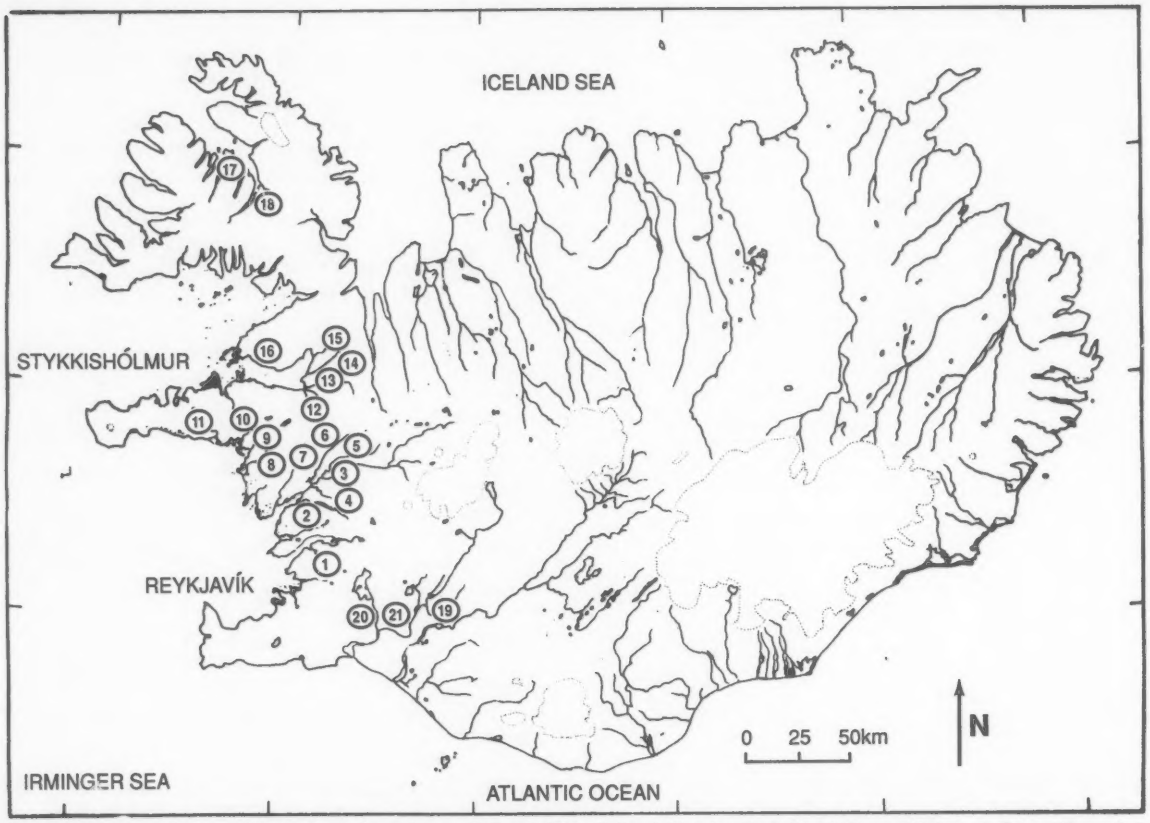

FIG. 1. Locations and rivers of Iceland mentioned in text. 1, Laxá í Kjós; 2, Laxá í Leirársveit; 3, Hvítá (Borgarfjördur); 4, Grímsá/Tunguá; 5, Thverá; 6, Nordurá; 7, Langá; 8, Álftá; 9, Hítará; 10, Haffjardará; 11, Straumfjardará; 12, Midá í Dölum; 13, Haukadalsá; 14, Laxá í Dölum; 15, Fáskrúd; 16, Flekkudalsá; 17, Laugardalsá; 18, Langadalsá; 19, Stóra-Laxá; 20, Sog; 21, Ölfusá–Brúará–Hvitá (combined).

effects of these fisheries on age structure of the salmon stocks are not known.

These net fisheries have been pursued throughout the duration of the useable angling records. It is possible that up to 50% of the returning run may be taken in these fisheries. The net fishery on Hvítá (Borgarfjördur) has a direct effect on subsequent angling catches in Hvítá, and the rivers Grímsá, Thverá, and Nordurá, which drain into Hvítá. Similarly, the net fishery on the Ölfusá-Brúará-Hvítá system has a direct effect on the angling catches in Sog and Stóra-Laxá. Although data are lacking, we assume that harvest emphasizes MSW salmon whenever possible. Results from these rivers should be interpreted with these precautions.

Details of the recreational fishery were provided by Gudjónsson (1978) and Scarnecchia (1983, 1984a). The number of rods per river is strictly regulated, and effort in general remains constant from year to year. The number of rods has, however, gradually increased at variable intervals of several years in most rivers (Scarnecchia 1984a). The effects of this increased effort on catch are not specifically known, although reported catches have risen on most rivers in recent years. In this paper, we assumed that catches were representative of abundance (as had been assumed in Scarnecchia 1983, 1984a, 1984b), at least over periods of adjacent years, and that ratios based on the number of grilse in one year to 2SW salmon the next year could be calculated with minimal bias from year-to-year effort changes. In some rivers, incompletely reported data, known effort changes, or other known biases for a given year made it impossible to compare one year's data with that of the previous or succeeding year. All such years known to us were deleted before any analyses. Data sets ranged from 9 to 39 yr (Table 2).

For the 21 rivers, we determined the number of years salmon had spent at sea by analyzing weight frequencies of rod-caught salmon separately by sex and calculating the number of fish of each age separately for each river and year. Each salmon was classified as a grilse (1SW), 2SW fish, or 3SW fish. Available scale samples indicated that MSW salmon included repeat spawners. Our analysis considered only grilse and 2SW salmon and included any repeat spawners which would have fallen into the appropriate weight frequency categories. We did not consider 3SW fish nor repeat spawners that fell into this weight category. Such fish were uncommon in the rivers. Because catches on all rivers were low, sexes were combined in our analyses to increase our sample sizes. Ratios of grilse in year x to 2SW salmon in year $x + 1$ (henceforth called ratios) were calculated and used in subsequent analyses.

The total West Greenland catch was used as a variable and the relationship investigated between the Greenland catch in year x and the grilse (year x) to 2SW (year $x + 1$) salmon ratios for the 21 rivers.

Sea Temperatures

Data on sea temperatures used were monthly mean surface values at Stykkishólmur, a long-standing and well-documented

TABLE 2. Years of data used in all analyses reported in this paper.

River	Years investigated
Laxá í Kjós	1949–50
	1953–56
	1959–82
Laxá í Leirársveit	1955–83
Hvítá (Borgarf)	1967–79
	1982–84
Grímsá/Tunguá	1972–84
Thverá	1956–73
	1976–82
Nordurá	1949–78
	1981–84
Langá	1965–84
Álftá	1965–84
Hítará	1961–62
	1965–84
Haffjardará	1969–84
Straumfjardará	1965–84
Midá í Dölum	1970–78
	1981–84
Haukadalsá	1948–60
	1963–84
Laxá í Dölum	1946–84
Fáskrúd	1949–84
Flekkudalsá	1961–82
Laugardalsá	1954–84
Langadalsá	1961–74
	1978–84
Stóra-Laxá	1974–78
	1981–84
Sog	1975–84
Ölfusá-Brúará-Hvítá	1964–84

station on Iceland's west coast. Monthly means were based on several, usually equally spaced measurements per month obtained through the Icelandic National Weather Office. Three different combinations of temperatures were analyzed against ratios. The first temperature variable (T1) was the sum of monthly mean temperatures from May to December (year $x - 1$), corresponding to the time when the smolts would enter the sea and begin rapid growth until their first winter at sea. The second variable (T2) was the sum of mean monthly temperatures from May (year x) to April (year $x + 1$), corresponding to the time from when the grilse would be returning to rivers to when the 2SW salmon would be returning. The third variable (T3) was the mean April–May (year $x - 1$) temperature, which was used to indicate sea conditions in spring and early summer, just before or during the time smolts would be entering the sea. This variable was selected because Peterman (1985) concluded that this period may be critical in influencing sea-age at maturity of Bristol Bay sockeye salmon and because Scarnecchia (1984a) and others have concluded that the time soon after smolts enter the sea is important in determining year-class strength.

Of all 485 possible available monthly mean temperatures from January 1945 to May 1985, only 4 values were missing. These four monthly values were estimated from the average of the 10 monthly means from surrounding years, 5 before and 5 after.

Statistical Analyses

Pearson correlation coefficients were calculated for comparing grilse catches as well as ratios among rivers. Linear, mul-

TABLE 3. Correlations among grilse catches for 21 Icelandic west coast rivers. Italicized data, $P < 0.01$; $^*P < 0.05$.

		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
Laxá í Kjós	A	—																				
Laxá í Leirársveit	B	*0.77*	—																			
Hvítá	C	0.10	0.54*	—																		
Grímsá	D	*0.60**	*0.83*	0.57*	—																	
Thverá	E	*0.59*	*0.57*	0.62*	0.58**	—																
Nordurá	F	*0.76*	*0.76*	0.52*	*0.78*	*0.73*	—															
Langá	G	0.52*	*0.82*	*0.71*	*0.80*	*0.74*	*0.73*	—														
Álftá	H	−0.10	0.04	0.31	0.23	0.36	0.15	0.39	—													
Hítará	I	*0.75*	*0.73*	0.30	*0.74*	0.51*	*0.59*	*0.63*	−0.03	—												
Haffjardará	J	0.55*	*0.62*	0.43	*0.70*	0.51	*0.84*	*0.70*	−0.17	*0.62*	—											
Straumfjardará	K	0.55*	*0.73*	0.36	*0.78*	0.27	*0.59*	*0.61*	−0.04	*0.85*	*0.65*	—										
Midá í Dölum	L	0.45	0.29	0.00	0.32	0.12	0.53*	0.22	−0.09	0.47	0.23	0.54*	—									
Haukadalsá	M	*0.79*	*0.73*	0.18	0.43	0.47	*0.73*	*0.59*	−0.01	0.51*	*0.61*	*0.58*	0.45	—								
Laxá í Dölum	N	*0.45*	*0.63*	0.27	0.32	0.27	*0.59*	0.28	−0.16	0.03	0.54*	0.25	0.14	*0.61*	—							
Fáskrúd	O	0.42*	*0.50*	0.50*	*0.73*	0.30	0.38	0.47*	−0.28	*0.32*	*0.76*	0.53*	*0.44*	*0.51*	*0.66*	—						
Flekkudalsá	P	*0.63*	*0.60*	0.31	0.49	*0.71*	*0.73*	*0.68*	−0.01	*0.71*	0.61*	0.48*	0.39	*0.53*	0.17	0.40	—					
Laugardalsá	Q	*0.52*	*0.64*	*0.61*	0.50	*0.74*	*0.57*	*0.79*	0.37	*0.60*	*0.62*	*0.56*	0.14	*0.56*	0.30	0.32	*0.63*	—				
Langadalsá	R	*0.59*	*0.55*	0.44	0.60*	0.52*	*0.67*	*0.74*	−0.06	*0.55*	*0.81*	*0.61*	0.45	*0.67*	0.39	*0.71*	*0.71*	*0.67*	—			
Stóra-Laxá	S	0.42	−0.08	−0.07	0.25	0.36	0.34	0.07	0.31	0.10	0.26	−0.15	0.29	0.10	0.12	−0.00	0.43	−0.02	0.16	—		
Sog	T	*0.77*	*0.90*	0.46	*0.74*	0.67*	0.56	*0.88*	0.28	*0.91*	0.72*	*0.82*	0.12	0.56	−0.30	0.36	0.72*	*0.74*	0.73	−0.01	—	
OBH	U	0.38	0.25	0.41	0.62*	*0.66*	0.44*	0.49*	0.42	0.53*	0.43	0.35	0.07	0.22	0.01	0.08	0.48*	0.43*	0.26	0.67*	0.64*	—

TABLE 4. Correlations among ratios for 21 Icelandic west coast rivers. Italicized data, $P < 0.01$; *$P < 0.05$.

		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
Laxá í Kjós	A	—																				
Laxá í Leirársveit	B	*0.57*	—																			
Hvítá	C	0.26	0.41	—																		
Grímsá	D	*0.86*	*0.89*	0.45	—																	
Thverá	E	0.47*	0.44*	0.39	0.71*	—																
Nordurá	F	0.47*	0.28	0.41	0.54	*0.89*	—															
Langá	G	0.20	*0.64*	0.47	*0.74*	0.46	0.56*	—														
Álftá	H	0.28	0.45	0.47	0.66*	0.61*	0.43	0.51*	—													
Hítará	I	0.49*	*0.74*	*0.63*	*0.87*	*0.63*	0.43	0.56*	0.52*	—												
Haffjardará	J	0.03	0.35	0.36	0.46	0.39	0.55*	0.47	0.14	0.56*	—											
Straumfjardará	K	*0.71*	*0.70*	0.44	*0.81*	*0.83*	0.78	0.52*	*0.58*	*0.68*	0.48	—										
Midá í Dölum	L	0.47	0.50	0.29	0.25	0.43	0.39	0.01	0.08	0.20	0.17	0.48*	—									
Haukadalsá	M	*0.77*	0.27	0.51*	*0.78*	0.36	0.43*	0.31	0.33	*0.71*	0.41	*0.66*	−0.00	—								
Laxá í Dölum	N	*0.48*	0.19	0.18	0.17	0.45*	*0.46*	0.11	0.55*	0.18	0.09	*0.58*	0.50	*0.49*	—							
Fáskrúd	O	*0.68*	0.37*	0.49	0.48	*0.55*	*0.52*	−0.04	0.35	*0.55*	0.13	*0.64*	0.54	*0.57*	*0.60*	—						
Flekkudalsá	P	0.31	*0.63*	0.46	*0.75*	*0.66*	*0.69*	*0.65*	0.35	*0.77*	0.43	0.52*	0.28	0.43	0.08	0.38	—					
Laugardalsá	Q	0.39*	*0.53*	0.43	*0.73*	0.21	0.12	0.34	0.51*	*0.60*	0.10	*0.62*	0.60*	0.30	0.35	*0.47*	0.33	—				
Langadalsá	R	−0.04	−0.21	−0.17	−0.15	0.17	−0.00	−0.22	−0.04	−0.15	−0.35	−0.20	0.15	−0.07	0.06	−0.05	0.31	−0.05	—			
Stóra-Laxá	S	0.52	0.71*	0.66	0.59*	0.86	0.72*	*0.81*	0.77*	0.63	0.58	0.56	0.20	0.48	0.10	0.07	0.84*	0.18	−0.03	—		
Sog	T	−0.34	−0.01	0.42	−0.04	0.08	0.37	0.13	0.13	0.08	0.27	0.06	0.17	−0.02	0.23	−0.16	−0.33	0.34	−0.82*	0.02	—	
ÖBH	U	0.43	*0.63*	0.33	0.66*	0.51*	0.53*	*0.63*	*0.58*	*0.70*	0.35	*0.60*	0.15	0.38	0.36	0.40	0.51*	0.37	−0.27	*0.89*	0.20	—

tiple, and stepwise regressions were used to identify if and how sea temperatures or West Greenland catch related to ratios from rivers. A $P < 0.20$ value was the criterion for entry into the stepwise model. Because both sea temperatures and salmon catch time series tend to be autocorrelated, residuals of linear relationships between variables entering the stepwise models were analyzed with a Durbin–Watson statistic for possible problems with autocorrelation that might produce misleading results (Dempson et al. 1986). Homogeneity of variance and normality of residuals, two other regression assumptions, were also evaluated in the stepwise models.

Results

Correlations between Grilse Catches and between Ratios

Annual grilse catches were strongly and positively correlated among the 21 rivers. Of the 210 correlations of grilse between pairs of rivers, 82 were positive and highly significant ($P < 0.01$), 33 were positive and significant ($P < 0.05$), and none were negative and significant at either 0.01 or 0.05 (Table 3). Correlations among ratios were less strong but similar. Of these 210 correlations, 50 were positive and highly significant ($P < 0.01$), 33 were positive and significant ($P < 0.05$), and 1 was negative and significant ($P < 0.05$; Table 4). For correlations between grilse and for those between ratios, the closer relationships were found for those rivers with some of the larger catches and more reliable catch records. For example, the Rivers Álftá, Hvítá (Borgafjördur), and Midá í Dölum showed poor relationships and have low catches and less complete records than most of the other rivers. Nearly all rivers had certain years (e.g. 1978) with unusually high ratios, even though all fishing, data collection, and data analyses were conducted independently for each river.

Trends in Grilse to Salmon Ratios over Time

Based on regression analyses, only two rivers, Laxá í Leirársveit and Langadalsá, had significantly increasing ratios over their time series (indicating fewer 2SW salmon relative to grilse; Table 5). Eighteen of the 21 rivers had coefficients of determination less than 0.09, which indicated that no long-term trends existed in the ratios. Several rivers, however, did have a series of particularly low ratios in the late 1940s and 1950s, before the expanded Greenland fishery (e.g. Fáskrúd, Laxá í Dölum, Thverá, and others; Fig. 2a–2e). Low ratios were also found for these and other rivers sporadically throughout the years that the West Greenland fishery was active.

Ratios before and during the West Greenland Fishery

Because the intense harvest of salmon off West Greenland began in the early 1960s and time series for Icelandic salmon catches are of moderate duration, ratios for years before the fishery began could be calculated for only eight rivers with the longest time series (Table 6). These rivers produced between 4 and 14 ratios before the West Greenland fishery began and 21 to 25 ratios during the fishery. All eight rivers had lower mean ratios before the fishery began than during the fishery. Results were highly significant (t-test; $P < 0.01$) for Laxá í Leirársveit, which also showed a highly significant increasing ratio with time. Three of the rivers (Laxá í Kjós, Thverá, and Nordurá) showed significantly higher ratios during the fishery than before it ($P < 0.05$; Table 6). Three other rivers (Haukadalsá, Fás-

TABLE 5. Coefficients of determination for relations of ratios versus year and for stepwise analyses of ratios (dependent variable) versus West Greenland catch and three sea temperature variables (T1, T2, and T3). Variables entered stepwise models at $P < 0.20$. *$P < 0.05$; **$P < 0.01$.

River	Ratio vs. year	Ratio vs. year, 1960 onward	Variables entering stepwise model	Significance of equation ($P < 0.05$)	Percentage of variation in ratios explained	Individual variables with significance of $P < 0.05$
Laxá í Kjós	0.02	0.08	T3(+)	Yes	17	T3(+)
Laxá í Leirársveit	0.14*	0.00	Green(+)	Yes	22	Green(+)
Hvítá	0.01	0.01	None	No	—	None
Grímsá	0.06	0.06	T1(+)	Yes	44	T1(+)
Thverá	0.00	0.02	Green(+), T2(+), T3(+)	Yes	32	None
Nordurá	0.02	0.08	T2(+), Green(+)	No	15	None
Langá	0.01	0.01	None	No	—	None
Álftá	0.00	0.00	T3(+)	No	20	None
Hítará	0.00	0.00	None	No	—	None
Haffjardará	0.05	0.05	T3(−)	Yes	35	T3(−)
Straumfjardará	0.17	0.17	T3(+)	No	12	None
Midá í Dölum	0.03	0.03	T2(−), Green(+)	No	47	None
Haukadalsá	0.02	0.10	T3(+)	Yes	15	T3(+)
Laxá í Dölum	0.02	0.07	T3(+), T2(−)	Yes	31	T3(+)
Fáskrúd	0.00	0.12	T3(+), Green(+)	Yes	43	T3(+), Green(+)
Flekkudalsá	0.02	0.02	None	No	—	None
Laugardalsá	0.00	0.00	T3(+)	Yes	15	T3(+)
Langadalsá	0.41**	0.41**	Green(−), T1(−)	No	12	None
Stóra-Laxá	0.03	0.03	None	No	—	None
Sog	0.00	0.00	None	No	—	None
OBH	0.07	0.07	T3(+)	No	12	None

krúd, and Laxá í Dölum) showed significantly higher ratios at $P < 0.10$. Nevertheless, despite this increase in mean ratios with the onset of significant fishing off West Greenland, there was no progressive increase in ratios as the fishery continued through the 1960s, 1970s, and 1980s. Only 1 of the 21 rivers, Langadalsá, showed significantly ($P < 0.05$) increasing ratios from 1960 forward, i.e. once the fishery had expanded (Table 5). In addition, there was no significant relationship between ratios and West Greenland catch for 20 of the 21 rivers ($P < 0.05$; Table 5).

Influence of Sea Temperatures and West Greenland Catches on Ratios

The mean April–May temperature (T3) was significantly ($P < 0.05$) positively related to ratios for five of the rivers: Laxá í Kjós, Haukadalsá, Laxá í Dölum, Fáskrúd, and Laugardalsá (Table 5; Fig. 3a–3c). For one river, Haffjardará, the mean April–May temperature entered negatively. For Grímsá, the total May–December sea temperature (T1) entered positively and significantly ($P < 0.05$).

For only one river, Laxá í Leirársveit, was the West Greenland catch significantly related to the ratios (Fig. 4). The relationship was positive, as hypothesized, and held for one of the two rivers that had shown increasing ratios over time (Table 5). In addition, the West Greenland catch entered the model significantly ($P < 0.05$) for Fáskrúd once the mean April–May sea temperature (T3) had entered the model (Table 5). These significant results for Greenland catch occurred with a frequency similar to that expected by chance.

Thirteen of the 21 rivers had no variables enter the stepwise models at the 0.05 level (Table 5). At lower levels of significance ($P < 0.10$ or $P < 0.20$), other variables entered the stepwise models (Table 5). Final stepwise equations for 9 of the 21 rivers were significant ($P < 0.05$). In three cases, the combinations of variables entering the stepwise models explained 43, 44, and 47% of the variations in the ratios, but most equations explained far less than half of the variations in the ratios (Table 5). Residuals of all the stepwise models listed in Table 5 were analyzed to check regression assumptions and to assess if autocorrelation problems existed. Of the 16 rivers that had variables enter stepwise models, 1 (Laxá í Kjós) had autocorrelated residuals ($P < 0.05$). Two rivers (Fáskrúd and Laxá í Dölum) had heteroscedastic residuals that might have called for a method to stabilize variance. The frequency of these problems was similar to that expected by chance.

Discussion

The effects of harvesting MSW salmon on age composition of Atlantic salmon stocks are manifested in at least three ways, each associated with increasingly intensive harvest. In Case 1, there is a shift toward younger fish spawning that results from direct, selective annual removal of MSW fish. This shift may be short term and need not drastically change the genetic composition of the next generation of spawners if the remaining MSW salmon are still numerous and are more effective at spawning and leaving offspring than are grilse.

In Case 2, greater depletion of MSW salmon results in a long-term intergenerational genetic change in the stock. In this case, higher frequencies of grilse spawn and leave offspring, which results in higher frequencies of grilse in succeeding generations.

In Case 3, harvest rates of MSW salmon may be so high that, in addition to genetic changes in the spawning stock, stream habitat for their offspring as parr may be underutilized. The reduced number of parr, which are already likely to be offspring of grilse, grow rapidly, which further increases their tendency to mature precociously as parr (for males) or as grilse rather than as MSW salmon. Case 3 is an indirect life history response by the stock to reduced optimal age at maturity as a result of higher harvest rates on adults and lower densities of parr in streams (Gibson 1978; Montgomery 1983; Caswell et al. 1987).

In the Icelandic stocks, we see possible evidence of Case 1, but no evidence of Case 2 or 3. The lack of significant declines

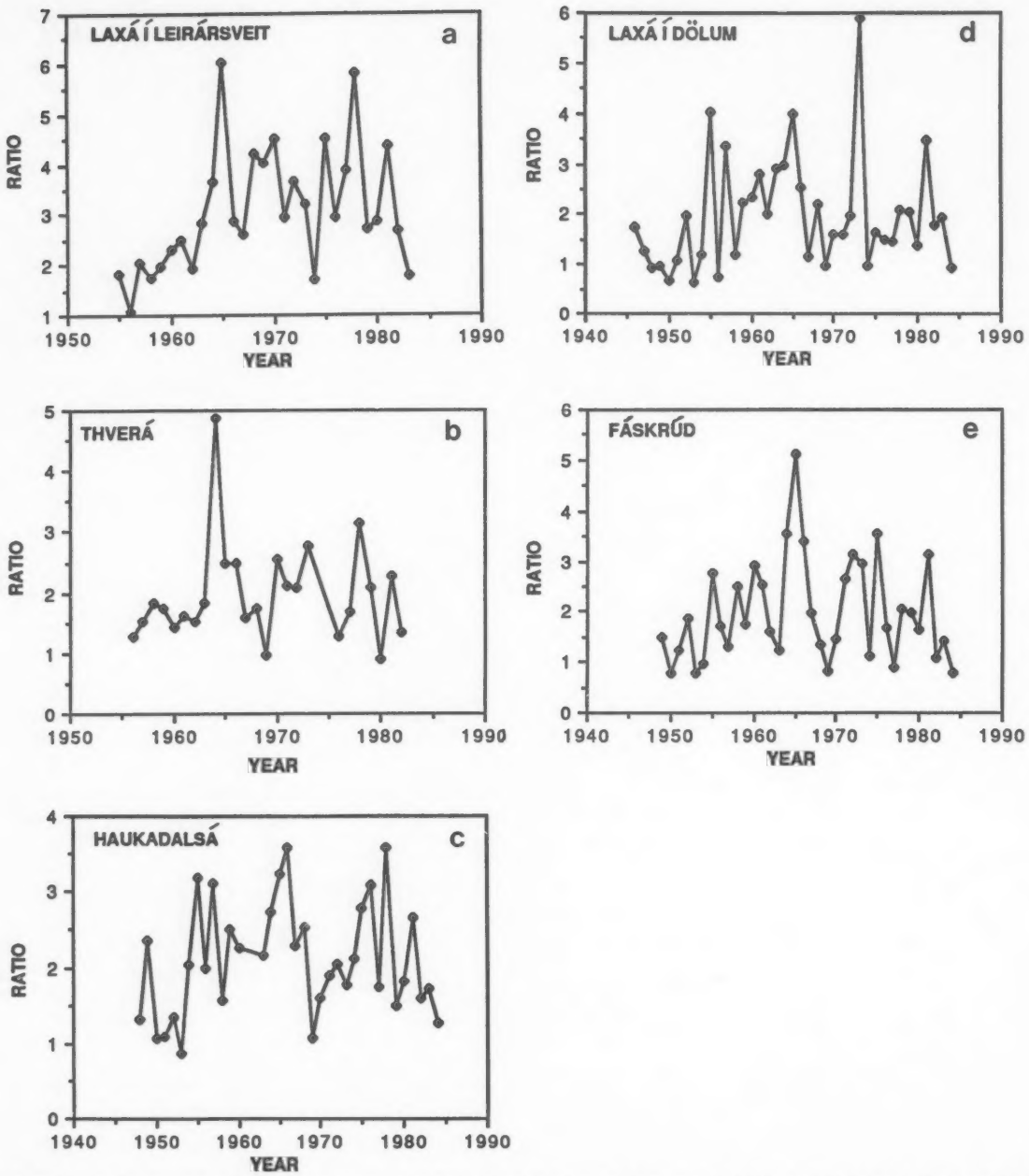

FIG. 2. Ratios of grilse (year x) to 2SW salmon (year $x + 1$) for (a) Laxá í Leirársveit, Ratio = $-0.65755 + 0.0540$(Year), (b) Thverá, Ratio = $1.7507 + 0.00310$(Year), (c) Haukadalsá, Ratio = $3.0745 - 0.0139$(Year), (d) Laxá í Dölum, Ratio = $0.96615 + 0.01519$(Year), and (e) Fáskrúd, Ratio = $1.5447 + 0.0065$(Year).

in ratios for all but 2 of the 21 rivers (Table 5) contrasted with results from the analyses of variance for pre- versus post-Greenland fishery ratios, where eight of eight rivers had higher ratios after the fishery had expanded in 1960. Although the ratios were higher for the eight rivers after the fishery began than before (as would be expected in Case 1), there was no progressive shift in age structure associated with the continuation, expansion, and later contraction of the fishery in the succeeding 25 yr. As indicated in Table 5, only 1 of the 21 rivers showed significantly increasing ratios from 1960 to 1985. If Case 2 or 3 had been occurring in the stocks, widespread significant increases in ratios would have been expected. It may be argued that since the West Greenland fishery harvested more potential MSW salmon in the middle portion of the time series than at

TABLE 6. Comparison of ratios before and after expansion of Greenland fishery. Number of years of data are in parentheses.

River	Before Greenland	During Greenland	Comparison of means Prob > T
Laxá í Kjós	2.11 (7)	2.97 (23)	0.05
Laxá í Leirársveit	1.73 (5)	3.35 (24)	0.01
Thverá	1.60 (4)	2.03 (21)	0.05
Nordurá	1.67 (11)	2.31 (23)	0.05
Haukadalsá	1.87 (12)	2.22 (23)	0.10
Laxá í Dölum	1.57 (14)	2.17 (23)	0.10
Fáskrúd	1.56 (11)	2.16 (25)	0.10
Laugardalsá	2.17 (6)	2.39 (25)	>0.10

either end, progressively higher ratios would not be expected. However, Greenland catch was poorly related to changes in ratios (Table 5), so the observed changes in ratios after 1960 must have been caused by other factors. These factors may be ecological or a result of subtle changes in harvesting patterns or data reporting in the riverine fisheries. But the changes evidently are not related to differences in mean April–May sea temperature, inasmuch as the temperatures before 1960 and after 1960 each averaged 3.5°C.

The long-term stability in ratios for all but 2 of the 21 rivers is in marked contrast with results from Atlantic salmon stocks in areas where significant oceanic and coastal fisheries are harvesting potential MSW salmon (e.g. Paloheimo and Elson 1974; Bielak and Power 1986). Ricker (1981) reported similar declines in mean sea-age of Pacific salmon. In Iceland, there is no directed oceanic or coastal fishery, and in-river rod fisheries harvest low to moderate numbers of returning salmon (total harvest rates most often 25–50%; Gudjónsson 1983). Rod fishing is undoubtedly less selective toward MSW salmon than large-meshed gill nets would be. Thus, even with harvest off West Greenland, numbers and genetic makeup of spawners and densities of parr in rivers are high enough to maintain historical percentages of MSW salmon. Case 2 and Case 3 effects from reduced frequencies of MSW salmon and reduced parr densities would be minimized. Perhaps the prudent harvests of Icelandic anglers have kept densities of spawners and parr up (as well as maintained different genotypes among the spawners and parr)

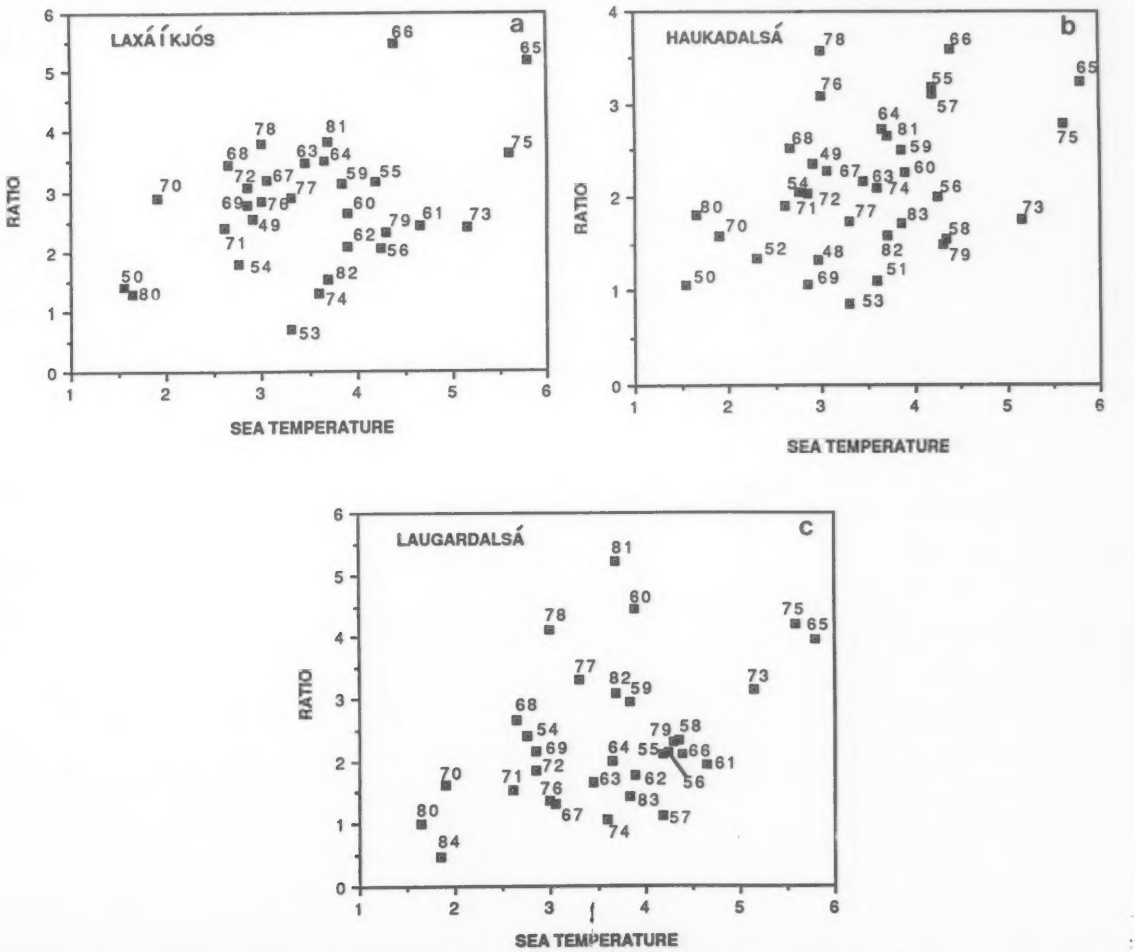

FIG. 3. Ratios versus mean April–May sea temperatures (year $x - 1$) for (a) Laxá í Kjós, Ratio = 1.2752 + 0.4264(Temp), (b) Haukadalsá, Ratio = 1.1008 + 0.2938(Temp), and (c) Laugardalsá, Ratio = 0.76428 + 0.44805(Temp).

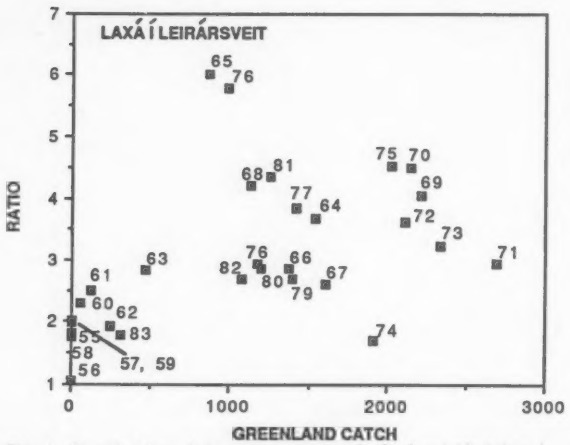

FIG. 4. Greenland catch in year x versus ratio for Laxá í Leirársveit. Ratio = 2.3177 + 0.00069(Catch).

and helped to maintain stock composition. Instead of declines in MSW salmon like those seen in many regions, anglers in Iceland can still catch the more desirable MSW salmon rather than just grilse.

In the absence of more detailed genetic data, the observed stability in sea-age composition serves as phenotypic evidence that serious total (oceanic plus riverine) overharvest of Icelandic salmon is not widespread. Excessive harvest from a combination of West Greenland or riverine fisheries over generations would have been expected to shift age composition toward more grilse. Declines in mean age at maturity from historical (i.e. lower harvest) frequencies should be used in other areas as an ersatz tool of genetic analysis to alert biologists to identify overharvest and possible loss of genetic diversity. Age structure relative to historical composition should be one of several indicators of sufficient genetic diversity and of a "safe biological limit" for Atlantic salmon that the International Council for the Exploration of the Sea's (1986) Working Group on North Atlantic Salmon has attempted to define.

However, close monitoring of certain rivers with increasing ratios (e.g. Laxá í Leirársveit and Langadalsá) is warranted. Even though the West Greenland fishery is not harvesting high percentages of potential MSW Icelandic salmon, nonsignificant relationship between West Greenland catch and ratios for a given stock does not imply that salmon of that stock are not being caught off West Greenland. In addition to the actual observed recoveries of tagged Icelandic salmon there, the variations in ratios produced by low harvest rates are often small relative to observed natural variations in the ratios resulting from differential annual riverine catch or abundance estimates. Because of noise in the ratios, they may be ineffective at detecting low or moderate harvest rates off West Greenland.

For example, if we assume constant natural mortality between the grilse and 2SW stages and a constant riverine fishery and consider a stock with a natural constant 1:1 grilse to salmon ratio, it would take a 50% harvest rate in the West Greenland fishery on the potential 2SW salmon eventually returning to the river for the ratio to rise to 2:1 (Fig. 5). Many Icelandic stocks exhibit substantial variation in ratios, e.g. Thverá, whose ratios vary from 0.90 to 3.13 (mean = 1.96; n = 25). Assuming that the observed mean pre-Greenland ratio for Thverá (1.6, n = 4) represented the natural situation, then a post-Greenland ratio of 2.4 might correspond to a harvest rate of 33% on 2SW salmon, and a ratio of 3.2 might correspond to a harvest rate of 50% (Fig. 5). Since 1960, Thverá has had 5 yr with ratios 2.4 or higher and no years with a ratio as high as 3.2. It is therefore unlikely that 2SW fish from Thverá are harvested at rates as high as 33–50%. Given the inherent annual variation in the ratios, however, a harvest rate below 33% is difficult to detect (Fig. 5). The observed annual variability in the ratios thus limits their applicability for indicating subtle effects of environmental factors. Some of this annual variability undoubtedly is noise — an artifact of the data collection and analysis procedures (e.g. classification into ages by weight-frequencies or variable annual effort).

If variations in environmental conditions as well as the substantial inherent variation in the catch data can be averaged over time and if only Case 1 effects (direct removal) are acting, it is possible to estimate what the average harvest rates off West Greenland would be to result in the observed changes in ratios before and after 1960. Expected harvest rates for the seven rivers with sufficient data and significantly higher mean ratios after 1960 varied from 16% of the MSW salmon that would have eventually been caught from Haukadalsá to 48% of the MSW salmon that would have been caught from Laxá í Leirársveit. Hypothetical mean harvest rates for the other five rivers were 29% for Laxá í Kjós (Fig. 6), 21% for Thverá, 28% for Nordurá, 27% for Laxá í Dölum, and 28% for Fáskrúd.

The close correlation between annual grilse catches in these rivers indicates to us that a widespread environmental factor (or factors) is strongly affecting the abundance of Icelandic salmon (Scarnecchia 1984a). Similarly, the significant correlations of ratios among rivers indicates that some large-scale environmental factor is responsible, even if this influence is small compared with the known close grilse-to-salmon relationship within stocks (Scarnecchia 1984b). Strong correlations between ratios do not imply an oceanic effect on age at maturity per se. The same correlations may result simply from similar conditions in freshwater during the presmolt period or from straying of salmon among groups of rivers. Our results do agree, however, with those of Peterman (1985), who found that mean age at maturity of Bristol Bay stocks of sockeye salmon of the same smolt class tended to vary in the same direction. Peterman (1985) also concluded that late ocean life probably was not a major source of variation in age at maturity. Our results also support that conclusion; in none of our 21 rivers was age at maturity significantly correlated with sea temperatures between the times of return of grilse and 2SW salmon to the rivers. If age at maturity is affected by oceanic variations, it evidently occurs during the time from when smolts leave the river to when grilse return.

If age at maturity of an individual fish can be influenced by oceanic conditions early in marine life, we would expect to see a relationship between sea temperatures early in marine life and ratios of that smolt class. Six of the 21 rivers showed a significant, positive relationship between pre-grilse sea temperatures (T1 or T3) and ratios. Although, at first inspection, this frequency is higher than would be likely to occur by chance, the correlation between ratios makes these results not strictly independent of each other. Our variable results among rivers underscore the importance of using several rivers in this sort of analysis. We thus have only suggestive evidence that oceanic conditions, as reflected in sea temperatures, affect the ratios. Such a relationship would occur if the warmer sea was indicative of better conditions for feeding, growth, and survival of

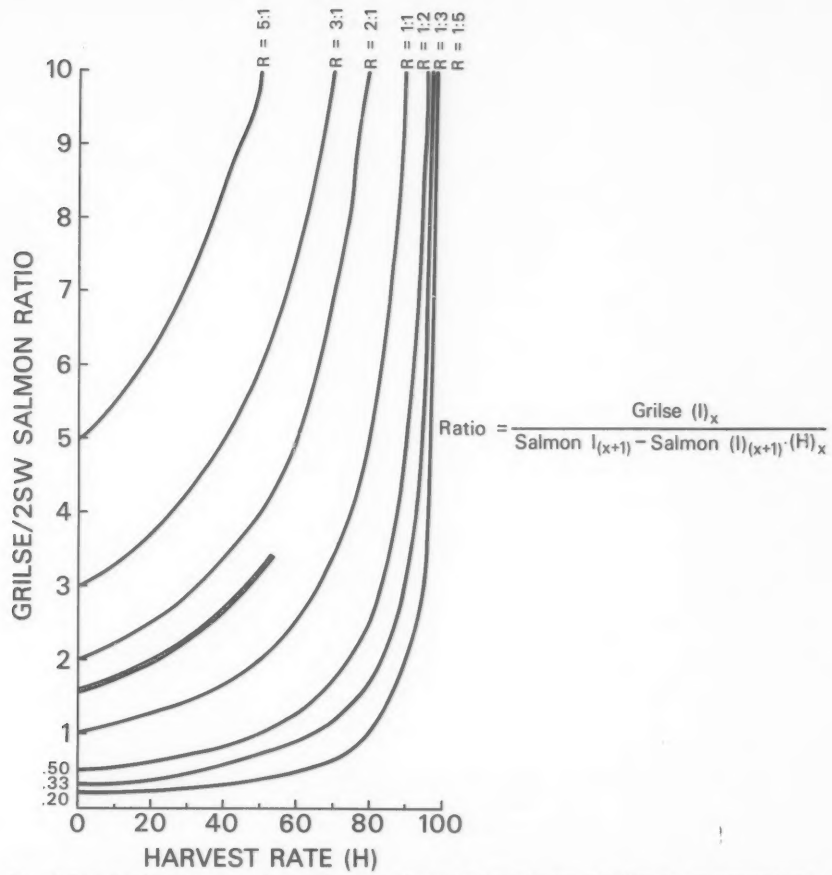

FIG. 5. Relationship between ratios and harvest rates for stocks with several different age compositions. Heavy, abbreviated curve is for Thverá assuming that the observed mean ratios before the Greenland fishery represented baseline ratio for the stock. See text for example.

smolts (Scarnecchia 1984a). If the smolts grew faster in their first year, relatively more of them might make a physiological decision (Thorpe 1986) to mature as grilse. In years of poorer growth, relatively fewer would mature as grilse, and relatively more MSW salmon would result.

Evidence of this process can be most easily seen from pen-rearing data for salmon (summarized by Dempson et al. 1986), where individual fish can be followed to maturation. Catch data and scale data, with the inherent biases of size-specific growth and mortality, merely confuse the issue. Dempson et al. (1986) cited numerous studies (e.g. Simpson and Thorpe 1976; Naevdal 1983; Gjerde 1984) indicating that marine growth in early-maturing fish was greater in the first year than in non-maturing fish, which is consistent with the positive relationship between sea temperature and ratios.

Dempson et al. (1986) questioned whether available evidence from wild salmon supported the findings of pen-rearing experiments. We believe, however, that reported results for wild fish do not contradict the results of pen-rearing experiments. Studying the relationship between growth rate and subsequent maturity *before the maturation occurs*, as in pen-rearing, is not comparable with studying the relationship based on scale samples from adult fish, i.e. *after the maturation occurs*. Assuming that the faster growing pen-reared fish do make a physiological decision to mature earlier, their growth slows relative to nonmaturing ones once they actually begin to mature, as energy is diverted into sex products. Thus, for wild fish, the initially faster growing individuals may in fact be smaller at time of first oceanic annulus formation, which would account for the smaller first-year growth increment in grilse than in MSW salmon in much of Gardner's (1976) scale data, as well as in the recalculated scale data of Dempson et al. (1986). The end products of the maturation process (scales of returning adult fish) may thus indicate that growth during the first year at sea was slower for fish destined to return as grilse, when the opposite was true.

Because we have not found evidence of excessive widespread harvest of Icelandic west coast salmon stocks by the West Greenland fishery, perhaps many MSW salmon from western Icelandic stocks feed in other areas and do not migrate to West Greenland. Mathisen and Gudjónsson (1978) suggested that most grilse from southwestern rivers might feed in the Irminger Sea but hypothesized that the 2SW salmon migrate either to West Greenland or toward the Faroe Islands. Perhaps many of the 2SW salmon have a localized migration pattern as well and remain in the Irminger Sea or off East Greenland (Ísaksson 1986). More tagging and recovery studies are needed to answer this question. Such studies are also needed to obtain direct evi-

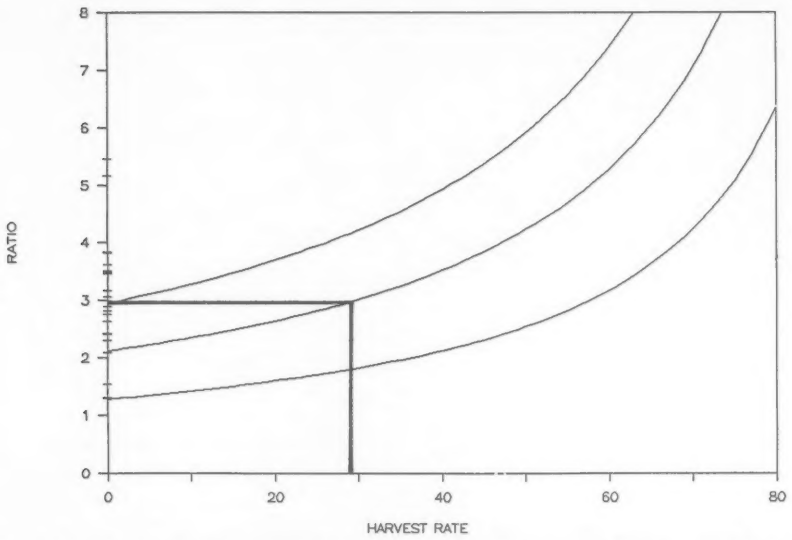

FIG. 6. Mean hypothetical harvest rate of 29% for would-be 2SW salmon off West Greenland for Laxá í Kjós. Annual ratios comprising the mean for the river are marked on the *y*-axis. Lower and upper curves are 95% confidence limits. Harvest rates mentioned in the text for the four other rivers were calculated analogously.

dence concerning harvest of Icelandic salmon off West Greenland.

Acknowledgements

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Tuna Purse-Seine Fishing with Fish-Aggregating Devices (FAD): Models of Tuna FAD Interactions

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Hilborn, R., and P. Medley. 1989. Tuna purse-seine fishing with fish-aggregating devices (FAD): models of tuna FAD interactions. Can. J. Fish. Aquat. Sci. 46: 28–32.

A model is proposed for the analysis of fishing tuna from fish-aggregating devices (FAD), and parameters for the model are estimated. Methods are described for determining the optimum number of FAD's to be deployed and the optimum number of vessels in a fishery. Field measurement of recruitment and loss of fish associated with FAD's is needed for adequate biological or economic analysis of FAD fishing. Results show that if recruitment to FAD's is proportional to the biomass not associated with FAD's, then increasing the number of FAD's beyond some limit will actually decrease total catch. Similar models could be used in any trap fishery, particularly for crabs or lobsters.

Un modèle est proposé ici pour l'analyse de la pêche aux thons avec des dispositifs de concentration des poissons. Des paramètres du modèle sont évalués. Diverses méthodes sont décrites pour déterminer le nombre optimal de dispositifs à déployer ainsi que le nombre optimal de navires dans une pêcherie. Des mesures sur le terrain du recrutement et de la perte de poissons associées à ces dispositifs de concentration des poissons sont nécessaires à toute analyse biologique et économique adéquate de la pêche avec ces dispositifs. Les résultats montrent que si le recrutement lié aux dispositifs de concentration est proportionnel à la biomasse non associée à ces dispositifs, l'augmentation du nombre de dispositifs au-delà d'une certaine limite diminuera en fait la prise totale. Des modèles semblables pourraient être utilisés dans toute pêcherie avec trappes, notamment les pêcheries de crabes et de homards.

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Many purse-seine fisheries for tuna rely on the tendency of yellowfin (*Thunnus albacares*) and skipjack tuna (*Katsuwonus pelamis*) to associate with floating objects or marine mammals. In the eastern Pacific, 33–65% of yellowfin tuna are captured in sets associated with marine mammals (IATTC 1982), and generally less than 10% with debris (Greenblatt 1979). In the western Pacific, porpoise schools play an unimportant role in purse seining, but approximately 75% of skipjack and yellowfin captured by U.S. purse-seine vessels in the western Pacific are associated with floating logs (Gillett 1986). Similarly, between 1982 and 1984, 45% of the tuna catch in the western Indian Ocean was associated with floating debris (Hallier 1985).

In the last 10 yr, man-made rafts, generally referred to as fish-aggregating devices or FAD's, have become very important in many fisheries, including artisanal and industrial tuna fisheries. Initially, most FAD's were moored reasonably close to shore and did not play a significant role for large purse seiners. Now, however, many U.S. and Japanese seiners deploy their own unmoored FAD's, and in several countries, local purse-seine vessels have deployed their own set of moored FAD's and fish them much like a trapline (Farman 1987).

The rapid growth of the Philippine tuna fishery is due primarily to widespread use of FAD's. This fishery involves 80 purse seiners from 100 to 1000 t and captured 242 000 t of tuna in 1983 (Floyd 1986), although not all of this was caught by seiners. The single most successful seiner in the western Pacific has been fishing primarily on FAD's in the Solomon Islands, and a single New Zealand purse seiner operated intermittently in Fiji for several years fishing on a string of FAD's it had deployed (Farman 1987).

Purse-seine operation on FAD's is reasonably straightforward. The boats generally make one set per day, usually at sunrise, and with luck capture the entire mass of tuna associated with a particular FAD. Vessels normally fish 20–25 d/mo. Vessels usually return to port for supplies, crew rest, etc., during the full moon. Catches range up to 50 t per set; the average for the vessel which operated in Fiji was about 10 t per set (Farman 1987). The Solomon Islands seiner averages 25 t per set. The set normally takes 2–4 h, with the rest of the daylight hours used in travelling to other FAD's and measuring number of fish with echo-location equipment. The vessels search for the FAD with the largest number of fish and then spend the night some distance from the target FAD, moving in at daylight to set the net. Occasionally the associated fish have left the FAD by morning, or weather conditions may prohibit successful fishing. Thus, successful sets occur on less than 100% of the days the vessel attempts to fish.

Within the economic zone of a given country, fisheries managers would like to know how the catch and profitability could

be expected to vary with different numbers of vessels and the number of FAD's each vessel deployed. Individual fishing skippers have developed some rules of thumb about optimal spacing of FAD's from their own experience, but as far as we are able to determine, there has been little systematic exploration of FAD spacing and frequency of fishing.

There have been two theoretical studies appropriate to analysis of FAD's. In the first, Clark and Mangel (1979) considered the interaction between tuna associated with marine mammals (the vulnerable population) and other tune not associated with marine mammals (nonvulnerable population). They considered how the exploitation rate on vulnerable fish would affect the dynamics of the population, given alterantive assumptions about exchange between the vulnerable and nonvulnerable populations. Samples and Sproul (1985) considered an explicit model of tuna dynamics associated with FAD's, for the purpose of economic analysis of an open access fishery using FAD's. This model differs from Clark and Mangel's (1979) primarily in that Samples and Sproul assumed that the recruitment rate to FAD's depends on the total population size, including fish both unassociated, and associated with FAD's. As we shall see, this makes a very important difference, although the data are not now available to discriminate between these competing hypotheses.

The purpose of this paper is to use a model similar to that of Clark and Mangel to see how FAD's and fishing vessels interact. We use estimates of the appropriate parameters to examine how catch and profitability are affected by number of vessels and number of FAD's. The results are useful for determining the types of questions to ask in an experimental program and provide interesting insight into optimal vessel and FAD deployment.

The Model

If we consider an area in which the biomass of tuna not attached to FAD's (B) is governed by a recruitment rate to the unattached population (R) independent of B, a mortality rate (m), and a loss rate (p) of fish to FAD's, then Equation 1 describes the rate of change of fish biomass unassociated with FAD's:

$$(1) \quad \frac{dB}{dt} = R - Bm - pNB + lA$$

where N is the number of FAD's in the area, A is the biomass of fish associated with FAD's, and l is rate of movement from the attached to the unattached populations. The above model assumes random encounters by individual fish with FAD's, so that the loss rate to FAD's is proportional to the number of FAD's (N) and the biomass of unattached fish (B).

Let the dynamics of fish associated with an individual FAD be governed by an input rate proportional to the biomass of unattached fish (B) and a loss rate proportional to the biomass of fish at the FAD. The biomass of fish at any FAD (Z) would follow the equation

$$(2) \quad \frac{dZ}{dt} = pB - lZ - mZ$$

where pB is the recruitment rate to the FAD, l is the loss rate to the unassociated population, and m is the mortality rate. If all fish are removed from a FAD when it is fished, and we call the time of fishing time 0, then the biomass at the FAD t days after it has been fished will be given by

$$(3) \quad Z_t = \frac{pB}{l+m}(1-e^{-t(l+m)}).$$

If there are N FAD's and the proportion of days on which the fishing vessel fishes is q, then each FAD will be fished N/q days after it was last fished. The abundance at each FAD when it is fished is therefore $Z_{N/q}$ (C) and the catch each time a FAD is fished will be

$$(4) \quad Z_{N/q} = C = \frac{pB}{l+m}(1-e^{\frac{-(l+m)N}{q}}).$$

Assume that each FAD is fished every N/q days and that a stable rotation of fishing is established between FAD's. This is slightly unrealistic, since vessels normally fish every day and then take a break, as discussed above. Therefore, if $q = 1$ for illustration, there will be one FAD fished 1 d previously, one FAD fished 2 d previously, etc. The biomass attached to FAD's will be therefore

$$(5) \quad \sum_{i=1}^{N} \frac{pB}{l+m}(1-e^{\frac{-(l+m)i}{q}}).$$

We assume that there are two costs to fishing, vessel costs and FAD costs. Total costs are therefore vessel costs plus the cost per FAD times the number of FAD's. The profits will be catch times price minus costs.

This model is essentially the same as Clark and Mangel's (1979) model A. The major differences are that we consider only one set per day to be possible, a difference between FAD's and marine mammals, and they did not consider that N (in our case FAD's; in theirs, number of porpoise schools) is a control variable. Our model differs from that of Samples and Sproul (1985) in that in our model the recruitment rate to FAD's depends upon B (unattached biomass) whereas in their model it depended upon the total population ($B+A$). They also did not consider the limitation of one set per day.

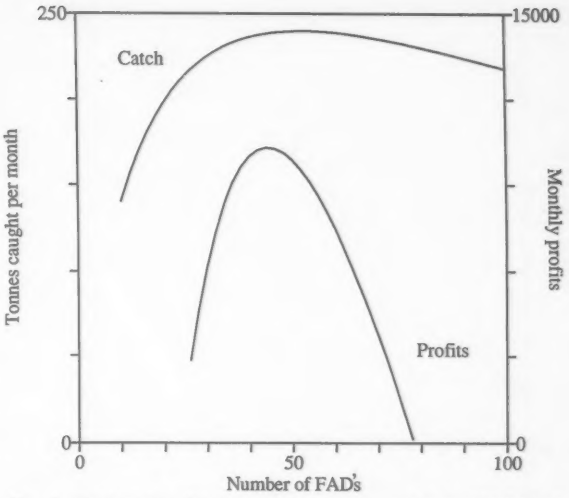

FIG. 1. Catch and profits for a single vessel operating in 10 000 km² as the number of FAD's is varied.

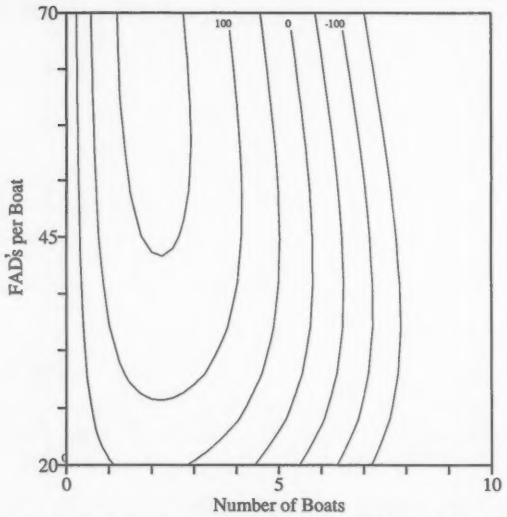

FIG. 2. Total catch and profit isoclines for 50 000 km² as the number of seiners and number of FAD's per seiner are varied.

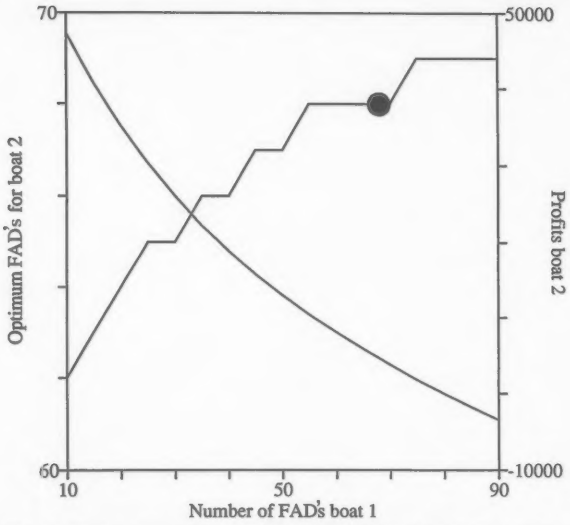

FIG. 3. Optimum number of FAD's deployed by boat 2 (increasing line), and profits realized by boat 2 (decreasing line), given the number of FAD's deployed by boat 1. Parameters for 20 000 km². The solid circle indicates the Nash equilibrium where the optimum number of FAD's for each player is the same.

A further difference between our model and both of the previous models is that we consider recruitment constant, they considered the implication of recruitment overfishing using the traditional Schaefer (1954) production model. A production model could be added to our model quite easily, but for the purposes of this paper we are concerned more with the implications of FAD deployment given constant recruitment. Certainly for skipjack tuna, there is no evidence from any fishery of recruitment overfishing. Floyd (1986) suggested that growth overfishing may have taken place on all species of tunas in the Philippine FAD fishery.

Parameter Estimates

We will use parameters appropriate to skipjack tuna in the western Pacific for a first examination of the model, although we will have to use higher recruitment and standing stocks in order to represent the fact that both skipjack and yellowfin are caught at FAD's, and the fishery would certainly not be profitable on either species alone. For initial discussion, let us consider an area of 10 000 km², which is roughly a 1° square. The mortality rate of skipjack is often considered to be about 20%/mo (Kleiber et al. 1983). Unfished biomass is assumed to be on the order of 0.1 t/km² for skipjack, averaged throughout the western Pacific. We will use 0.25 t/km², or 2500 t/10 000 km², to account for the combination of skipjack and yellowfin and the fact that fishing normally takes place in locations of higher than average abundance. Therefore the recruitment rate for 10 000 km² will be 500 t/mo.

To estimate p, the probability that an individual fish will encounter a FAD and stay with it, we used the following calculations. Assume that a tuna travels at 5 km/h, 12 h/d, 30 d/mo and would detect a FAD within 25 m on either side. This means that the area searched per month is 90 km² or 0.009 of the area under consideration. For daily calculations, use 0.009/30 or 0.0003. The loss rate from FAD's, l, was calculated so that the standing stock of tuna under a FAD when unfished for a long time would be roughly 40 t. At equilibrium, the loss rate from a FAD ($(l+m)Z$) will be exactly equal to the recruitment rate to the FAD (pB). Using 2500 t as the unfished biomass, $p = 0.0003$, and $Z = 40$, we estimate l as roughly 0.012/d.

To estimate costs, we assumed that a vessel catching 200 t/mo and receiving $500/t would just pay vessel costs. This means a vessel costs $100 000/mo for all fixed and variable expenses other than FAD's. A common price for moored FAD's is $5000 with a life expectancy of 2 yr. This produces a monthly cost of roughly $\$200 \cdot FAD^{-1} \cdot mo^{-1}$.

All of the above estimates are extremely rough, and our major purpose is to examine the behavior of the model given some potentially realistic parameter estimates. Cost data for specific fisheries would normally be available to people involved in fisheries development, but the biological data and FAD data are going to be much more difficult to obtain. We discuss these problems in a later section.

Bard et al. (1985) and Hallier (1985) have presented data for the Atlantic and Indian oceans on repeated sets at the same floating debris. They found that often the catch on second and subsequent days was nearly as large as on the first day. This contrasts with the echo-location information in the western Pacific which indicates that the entire school under a FAD is removed by a successful set of the net.

Results

We numerically solved Equations 1–5 for conditions during a stable rotation. Figure 1 shows how catch and profit depend upon the number of FAD's for a single vessel operating in 10 000 km². Profits are maximized with about 40 FAD's, which corresponds to one FAD every 250 km², or a mean distance between FAD's of 15.8 km. The seiner which operated in Fiji used a general rule of keeping FAD's no closer than 16 km, in such close agreement with our calculation that it must be pure chance.

There is one unexpected result in this figure: the catch drops off when many FAD's are deployed. Our intuition suggested that catch should asymtotically approach a maximum as the number of FAD's were increased. However, examination of the model reveals that the saturation level of a FAD depends upon the rate of arrival at the FAD, which is pB. As more FAD's are deployed, B will be smaller because more fish will be associated with FAD's. Therefore, as the number of FAD's increase, the maximum number of fish at a FAD will decrease, and catch, which will essentially be N/q times the FAD saturation level, will decrease at high FAD numbers.

Maximization of profits may require more than one vessel, and to investigate this we looked at a larger area, 50 000 km^2. To adjust the parameters, we multiplied the recruitment rate R by 5 and divided the probability of encountering a FAD "p" by 5. Figure 2 shows the profit isoclines for this analysis. Profits are maximized by two vessels operating 55 FAD's each.

We can examine the game-theoretic potential of a two-player game by assuming that one boat (boat 1) deploys a certain number of FAD's and looking at the optimum number of FAD's deployed by the second boat (boat 2) in response (Fig. 3). Parameters in this case were for 20 000 km^2. The fewer FAD's deployed by boat 1, the lower the optimum number of FAD's deployed by boat 2, and the higher boat 2's profits. This type of curve is often called a "reaction" curve, specifying how boat 2 should react to a decision by boat 1. If we start at some point, assume that boat 1 deploys 25 FAD's, and then have boat 2 make its choice, which would be about 65 FAD's, we can then reverse roles and ask what boat 1 would then do, since we assume that costs are equal and the game is perfectly symmetrical. Once boat 2 had deployed 65 FAD's, boat 1 would then deploy about 68. The game would continue until it reached an equilibrium, which in this case would be 68 FAD's each. This point is known as the "Nash equilibrium" (the solid circle in Fig. 3), and in this situation each player would stay with 68 FAD's. Both players would be losing money (negative profits).

This is somewhat surprising, since the normal "open access" fishery results in both players expanding until profits are zero. In this two-player game, however, if we were at a point where both players had zero profits, each individual player would actually increase his profits if he added a few more FAD's, assuming that the other player did not respond by adding more FAD's. Thus the dynamics of the two-player game are different, and economically worse than the strict open access case.

Discussion

The major benefit from our model is that it clarifies the kinds of data that are needed to understand the consequences of alternative vessel and FAD deployments. Data on how fish accumulate at FAD's would be a top priority. These data could be collected by measuring the number of fish at several FAD's over time, to see if fish do accumulate in a fashion similar to the model we proposed, or whether individual schools of fish arrive in a more or less random pattern. The difference between the apparently small proportion of fish taken by each set of the net in the Atlantic and Indian oceans (Bard et al. 1985; Hallier 1985) and the western Pacific FAD fishing, which suggests that all fish at a FAD are taken with each set, needs to be resolved. Daily acoustic soundings at a number of FAD's or logs would resolve this question. It is also important to know if the loss process is similar to the form we chose, or perhaps it is density dependent, with fish more likely to leave a FAD when there is a large accumulation. Only a field program could resolve these questions.

We can use the results of Fig. 2 to examine the potential dynamics of FAD deployment fisheries. If a single owner/operator had an exclusive property right to an area, then he would choose the number of FAD's to deploy based on his objective. If a government or corporation was in charge of the fishery, they would presumably choose the appropriate mix of number of vessels and FAD's per vessel to meet their objective. We can see from Fig. 2 that profit maximization would dictate a fleet of two vessels with 55 FAD's each whereas catch maximization subject to making zero profit would dictate a larger fleet with fewer FAD's per boat.

If the fishery was open access, then we would expect vessels to continue to enter the fishery until no profits could be made and to add FAD's to each vessel until profits went to zero. We saw from Fig. 3 that in a competitive situation the economic equilibrium for two boats would be to have so many FAD's that both players were making negative profits. This suggests that management agencies should consider regulating the number of FAD's each vessel is allowed, and the model would be used for making assessments of the appropriate number.

Although we developed the model to represent a vessel running a trapline of moored FAD's, it would seem appropriate for vessels operating on the high seas as well. The underlying dynamics are the same: free-floating FAD's would attract fish and the vessel would be running the FAD's much like a trapline. However, there would presumably be the opportunity to fish on other floating objects as well as make sets on schools of tuna unassociated with floating objects.

There are a number of obvious next steps to the analysis of tuna FAD models. One is to consider explicitly the spatial distribution of FAD's and the associated search time. In practice, the vessels check a number of FAD's each day and set on the one that has the most fish. If there was a stochastic arrival and departure model, the consequences of not being able to check every FAD every day might be quite interesting. The stochastic problem would then be quite similar to many optimal foraging problems, with a rather specific renewal process.

Alternative models of attraction and loss from FAD's could be examined, in particular models that assume that fish come and go in large units such as schools. The dynamics of games between multiple boats, with each one deciding when it was advantageous to place more FAD's, would also be well worth examining.

The model, as we presented, can equally well represent other types of fisheries, in particular trap fisheries. If we eliminate the loss term from the FAD's, then we have a model of crab traps, with each crab having a probability of entering a specific trap each unit of time. Considerations of crab trap deployment (e.g. Miller 1983) ignore the impact of a large number of pots reducing the recruitment rate to the crab pots. The model is structurally similar in that each vessel can only check so many traps per unit of time, and there are two dimensions to fishing effort, vessels and traps per vessel.

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Role of Exposure Duration in Hydrogen Ion Toxicity to Brook (*Salvelinus fontinalis*) and Rainbow Trout (*Salmo gairdneri*)[1]

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Acidification of streams and rivers associated with rainstorm or snowmelt events is often episodic as are many environmental introductions of toxic substances. We examined the toxicity of continuous or intermittent exposures to sulfuric acid (H^+) to brook trout (*Salvelinus fontinalis*) embryos, alevins, and fry. Acute toxicity tests were conducted with juvenile rainbow trout (*Salmo gairdneri*). These studies permitted evaluation of key components of intermittent exposures (toxicant concentration, exposure duration, and recovery period) on mortality, reduced growth, and perturbed electrolyte balance. Lethality of H^+ markedly changed with developmental stage of brook trout. Resistance of the chorion to H^+ penetration probably protected embryonic fish, while hatching and onset of active swimming exacerbated H^+ toxicity. Response surface methods demonstrated that between pH 4 and 7, time–concentration relationships for H^+ toxicity were greatly influenced by exposure duration and peak concentration but little by length of recovery period. Daily pulses at pH 4 with duration as short 4.5 h produced marked mortality after 90 d. This did not occur after 4–60 d of testing. Whole-body Na^+, K^+, and Ca^{2+} concentrations of brook trout were negatively correlated with mean H^+ concentrations after 90 d of exposure. Cation depletion appeared to be a more sensitive index of chronic, sublethal H^+ toxicity than reduced growth.

L'acidification des cours d'eau associée aux précipitations ou à la fonte des neiges est souvent épisodique, comme le sont de nombreux phénomènes d'introduction de substances toxiques dans l'environnement. Nous examinons ici la toxicité d'exposition continue ou intermittente d'embryons, d'alevins et de truitelles de truites (*Salvelinus fontinalis*) de ruisseau à l'acide sulfurique (H^+). Les tests de toxicité aiguë ont été effectués sur des truites arc-en-ciel (*Salmo gairdneri*) juvéniles. Ces études ont permis d'évaluer l'effet des principaux paramètres des expositions intermittentes (concentration de produits toxiques, durée d'exposition et période de récupération) sur la mortalité, la réduction de la croissance et la perturbation de l'équilibre électrolytique. La létalité de H^+ variait de façon marquée selon le stade de développement de la truite de ruisseau. La résistance du chorion à la pénétration de H^+ protégeait probablement le poisson à l'état embryonnaire, tandis que l'éclosion et le début de l'activité de nage exacerbent la toxicité de H^+. Les méthodes de surface de réponse ont démontré qu'à un pH de 4 à 7, les rapports temps–concentration pour la toxicité de H^+ étaient grandement influencés par la durée de l'exposition et la concentration de pointe, mais peu par la durée de la période de récupération. Des injections quotidiennes d'une durée aussi brève que 4.5 h à un pH de 4 ont provoqué une mortalité marquée après 90 d. Ce phénomène ne s'est pas produit après 4–60 d d'essai. Les concentrations de Na^+, K^+, Ca^{+2} dans tout le corps de la truite de ruisseau étaient en corrélation négative avec les concentrations moyennes de H^+ après 90 d d'exposition. L'épuisement des cations a semblé un indice plus sensible de la toxicité chronique sublétale de H^+ que la réduction de la croissance.

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Transient elevations of stream water acidity and metal concentrations are associated with acid precipitation events (Driscoll et al. 1980; Gunn and Keller 1984). There has been little research on toxicity of episodic exposures of organisms to environmental contaminants. As indicated by earlier work with copper (Ingersoll and Winner 1982; Seim et al. 1984), with the insecticide fenvalerate (Curtis et al. 1985), and with acidic aluminum solutions (Siddens et al. 1986), extrapolation from continuous to intermittent exposures is inadequate for establishing regulations. This is of particular concern because most laboratory toxicity tests are conducted with con-

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tinuous exposures to constant concentrations or another simplified dosing regime. We investigated the toxic effects of various temporal exposure patterns to a major constituent of atmospheric pollution, sulfuric acid (H^+), in a 90-d embryo/larval test with brook trout (*Salvelinus fontinalis*) and acute toxicity tests with rainbow trout (*Salmo gairdneri*). Comparisons of toxicity for exposure regimes based on mean concentrations over entire experiments, or key components of intermittent exposures (peak concentration, exposure duration, recovery period), were made.

Time–concentration profiles for transient, precipitation-associated H^+ elevations in surface waters occur as two major classes of events. During snowmelt, pH reductions often persist for weeks (Jeffries et al. 1979; Heniksen 1976), while duration of rainstorm-associated events is more typically hours (Johannessen and Joranger 1976; Lynch et al. 1986). Snowmelt might produce a single major annual H^+ pulse, while frequency of rainstorm-derived H^+ will vary with season and regional climate. The magnitude of H^+ pulses can also vary greatly. Drops of about 2 pH units occurred in Scandinavian waters following snowmelt (Heniksen 1976) and rainstorms (Johannessen and Joranger 1976). We designed laboratory H^+ exposure regimes to examine the roles of exposure and recovery period durations in toxicity of "rainstorm-like" events and sensitivity of developmental stage in toxicity of "snowmelt-like" events. Given the infinite number of time–concentration profiles possible for field situations, we focused our attention on key elements of intermittent toxicant exposures: concentration, duration, recovery period, and relative sensitivities of life stages.

Methods

Exposure Apparatus

A gravity-fed flow-through dilutor described in Siddens et al. (1986) was used in all experiments. Water chemistry was controlled to maintain conditions representative of acid-sensitive (low hardness) natural waters. All test water was procured from a blend of untreated well water and well water softened by reverse osmosis to maintain total hardness at 8.0–10.0 mg/L and calcium at 1.7–2.8 mg/L. Details on the chemistry of water produced by this system and evidence that respective concentrations of cadmium, copper, and zinc were below 0.5, 2.5, and 5.0 ppb were published (Cusimano et al. 1986). Water temperature was between 10.0 and 13.0°C.

Embryos and alevins until onset of active swimming ("swim-up" stage) were held in polypropylene Buchner funnels described by Seim et al. (1984) at a flow of 300 mL/min ± 10%. At 62 d into the study, actively swimming fish were transferred to 60-L aquaria containing 30 L of water at a flow of 500 mL/min ± 10% and fed 10% body wt/day of Oregon Test Diet.

Blended water was acidified to pH 4.0 with sulfuric acid using a Beckman automatic titrator. Proportional dilutions of pH 4.0 and control water were mixed in a gravity flow system with manual valves to obtain and maintain target pH within 0.08 unit. The pH was recorded four times daily in constant-exposure tanks to determine peak/mean concentrations. Intermittent cycles were recorded with a Superscribe 4900 series strip chart recorder attached to an Orion pH meter equipped with a Ross combination electrode. The 90-d mean H^+ concentrations ($[H^+]$) were estimated by planimetry, while peak concentrations were taken directly from the time–concentration curves.

Ninety-Six-Hour (Acute) Toxicity

Juvenile (1.4–1.8 g) Shasta strain rainbow trout, donated by Department of Food Science, Oregon State University, were used in acute toxicity studies. Fifteen fish were exposed in 60-L aquaria containing 30 L of water at pH 6.8 (control), 4.35, 4.15, 4.0, or 3.80 continuously for 96 h or to pulses of pH 3.70, 3.80, 4.0, 4.15, or 4.35 for periods varying from 12 to 48 h. Between acidic pulses, exposure tanks received pH 6.8 water. Water flow rates of 600 mL/min were maintained at all times. Mortalities were recorded daily and LC50's calculated as detailed below.

Ninety-Day Toxicity

Table 1 details the exposure regimes used in a 90-d embryo/larval test conducted with brook trout. For a given regime, peak $[H^+]$'s were indistinguishable in funnels and aquaria. Time-weighted averages for both types of exposure chambers were used to calculate 90-d mean $[H^+]$'s. Time–concentration curves for intermittent exposures to copper (Seim et al. 1984), fenvalerate (Curtis et al. 1985), and H^+ plus aluminum (Siddens et al. 1986) were previously published. As in these cases, $[H^+]$'s over time were not square-wave events. Peak $[H^+]$'s in aquaria occurred after about 2, 2.5, and 3.5 h in respective pH 4.0, 4.5, and 5.0 regimes. Return to pH 6.8 after termination of H^+ introduction took 1.5 h in pH 4.0 regimes and about 1 h in pH 4.5 and 5.0 regimes. Similarity of time–$[H^+]$ curves to square-wave events increased with length of exposure duration as reflected in relationships between percent of time fish were exposed to H^+, peak $[H^+]$, and mean $[H^+]$ for each regime (Table 1).

Brook trout eggs were obtained from Wizard Falls Hatchery, Oregon Department of Fisheries and Wildlife. Spawning and egg fertilization were conducted at the hatchery where water pH was 7.1. Embryos were then transferred to the Western Fish Toxicology Laboratory, USEPA, Corvallis, OR, and approximately 250 embryos were placed in exposure chambers within 4 h of fertilization. Each of 54 chambers (two per exposure regime) were covered with rigid, black plexiglass to exclude light. Exposures of embryos to H^+ for the first 14 d after fertilization were started 1 h after their transfer to exposure chambers (day 0 of the test). Repeated short-duration exposures of 0.2 d H^+/0.8 d control water (0.2–0.8), 1 d H^+/7 d control water (1–3), were started the next day (day 1 of the test). A series of 4 d H^+/4 d control water exposures (4–4) were started on day 1, 2, or 3 of the test.

Five fish were removed from each exposure chamber (10 per regime) 2 wk after hatching and at 2-wk intervals thereafter. Any yolk was removed and dry weights were determined as previously described (Seim et al. 1984). After 60 and 90 d of exposure(s), two additional fish were removed from each exposure chamber (four per regime) and analyzed for Na^+, K^+, and Ca^{2+} to assess osmoregulatory competence. Whole fish (including yolk when present) were dissolved in nitric acid, as described by Seim et al. (1984), and analyzed by atomic absorption spectrometry. Samples were compared with National Bureau of Standards bovine liver processed in a like manner which verified greater than 98% efficiency of analysis.

Data Analysis

Ninety-six-hour LC50 estimates were made by the method of Hamilton et al. (1977). Percent mortality for the embryo/

TABLE 1. Time and concentration components of H^+ exposures which were systematically varied over a 90-d embryo/larval experiment with brook trout.

Regime abbreviation[a]	H^+ pulse duration (d)	Recovery period (d)	Percent time exposed[b]	Peak [H^+] (μg/L)[c]	Mean [H^+] (μg/L)[d]
0.2–0.8 (pH 4.0)	0.2 (4.5 h)	0.8 (19.5 h)	19.0	94.0	13.8
0.2–0.8 (pH 4.5)	0.2 (4.5 h)	0.8 (19.5 h)	19.0	26.0	3.4
0.2–0.8 (pH 5.0)	0.2 (4.5 h)	0.8 (19.5 h)	19.0	7.7	1.0
1–7 (pH 4.0)	1.0	7.0	12.5	110.0	14.8
1–7 (pH 4.5)	1.0	7.0	12.5	32.3	2.7
1–7 (pH 5.0)	1.0	7.0	12.5	8.5	1.1
1–3 (pH 4.0)	1.0	3.0	25.0	114.5	28.2
1–3 (pH 4.5)	1.0	3.0	25.0	20.4	4.9
1–3 (pH 5.0)	1.0	3.0	25.0	5.5	1.4
4–4 (pH 4.0)	4.0	4.0	50.0	110.0	50.2
4–4 (pH 4.5)	4.0	4.0	50.0	32.3	15.7
4–4 (pH 5.0)	4.0	4.0	50.0	8.8	4.4
Days 1–14 (pH 4.0)	14.0	50.0	16.0	103.7	16.3
Days 1–14 (pH 4.5)	14.0	50.0	16.0	46.9	7.5
Days 1–14 (pH 5.0)	14.0	50.0	16.0	10.1	1.8
Days 15–28 (pH 4.0)	14.0	36.0	16.0	103.7	16.3
Days 15–28 (pH 4.5)	14.0	36.0	16.0	46.9	7.5
Days 15–28 (pH 5.0)	14.0	36.0	16.0	10.1	1.8
Days 29–43 (pH 4.0)	14.0	22.0	16.0	103.7	16.3
Days 29–43 (pH 4.5)	14.0	22.0	16.0	46.9	7.5
Days 29–43 (pH 5.0)	14.0	22.0	16.0	10.1	1.8

[a]This notation reflects the ratio of H^+ pulse to recovery period duration in the repetitive cycle regimes (first four rows of table) or the interval of days after fertilization (day 0) during which a single, 14-d H^+ pulse was introduced.

[b]Proportion of total experiment during which a group was exposed to elevated [H^+].

[c]Mean maximal [H^+]'s measured in various regimes over the course of the experiment. These were determined as maximal depression of time–pH curves during pulses transformed to [H^+].

[d]Average [H^+]'s for various regimes over the entire experiment. After conversion of pH to [H^+], areas under time–[H^+] curves were integrated for calculation of regime means.

larval test was calculated from survivorship corrected as previously reported (Seim et al. 1984). Body weight data were analyzed by one-way analyses of variance followed by the Tukey test for comparison of individual means. Significance was at $P < 0.05$. One-way analyses of variance and an exponential regression model were applied to whole-body Na^+, K^+, and Ca^{2+} data for brook trout. Response surface methods were applied to evaluate relationships between exposure pH, exposure duration, recovery period, and mortality (Carter et al. 1985). The form of the mathematical model was

$$P = \frac{1}{[1+e^{-(B_0+B_1X_1+B_2X_2+B_3X_3+B_{12}X_1X_2+B_{13}X_1X_3+B_{23}X_2X_3)}]^{-1}}$$

where X_1 = duration of exposure in days, X_2 = recovery duration in days, X_3 = pH, B_0 = unknown constant associated with probability of survival under baseline condition, B_1 = unknown constant associated with the effect of exposure, B_2 = unknown constant associated with the effect of recovery duration, B_3 = unknown constant associated with the effect of pH, B_{12} = unknown constant associated with the interaction between the duration of exposure and recovery duration, B_{13} = unknown constant associated with the interaction between the duration of exposure and pH, and B_{23} = unknown constant associated with the interaction between the duration of recovery and pH.

Results

Ninety-Six-Hour Toxicity

Juvenile rainbow trout died only at extremely high [H^+] in our 96-h test (Table 2). When the proportion of test time exposed was constant (50%), an acid cycle duration of 2 d was more toxic than 0.5 or 1 d. Almost no mortality occurred with daily 4.5-h pulses of H^+ (data not presented). Thus in the acute situation, a threshold exposure duration for H^+ was apparent.

TABLE 2. Influence of continuous and various intermittent exposure regimes on acute lethality of sulfuric acid to juvenile rainbow trout.

Exposure regime	Percent time exposed	96-h LC50 (95% confidence limits for mean H^+ concentration)
Continuous[a]	100	65.1 – 84.9
Daily 12-h H^+ pulse[b]	50	52.7 – 57.8
Alternate 24-h H^+ pulses[c]	50	46.8 – 55.8
Single 48-h H^+ pulse[d]	50	35.8 – 42.3

[a]Control water of pH 6.8 and acidified waters of pH 4.35, 4.15, 4.0, and 3.8 were used. Respective mean [H^+]'s were 0.2, 46.0, 67.0, 87.6, and 144.7 μg/L.

[b]Control water was supplied to aquaria between acidic pulses with minimal pH's of 3.70, 3.78, 3.89, and 4.06. Respective mean [H^+]'s were 71.3, 61.3, 45.4, and 19.9 μg/L.

[c]Control water was supplied to aquaria between acidic pulses with minimal pH's of 3.78, 3.92, 4.02, and 4.26. Respective mean [H^+]'s were 85.5, 77.1, 56.0, and 24.1 μg/L.

[d]Control water was supplied to aquaria between acidic pulses with minimal pH's of 3.76, 3.86, 3.96, and 4.26. Respective mean [H^+]'s were 83.1, 67.2, 51.7, and 25.8 μg/L.

Ninety-Day Toxicity

Continuous 90-d exposure of brook trout embryos and larvae to H^+ at pH 4.5 and 4.0 sharply decreased survival (Fig. 1). Survival of embryos for 10 d after fertilization was 100% at pH 5.0 (10 μmol H^+/L), dropped to about 12% at pH 4.5 (32

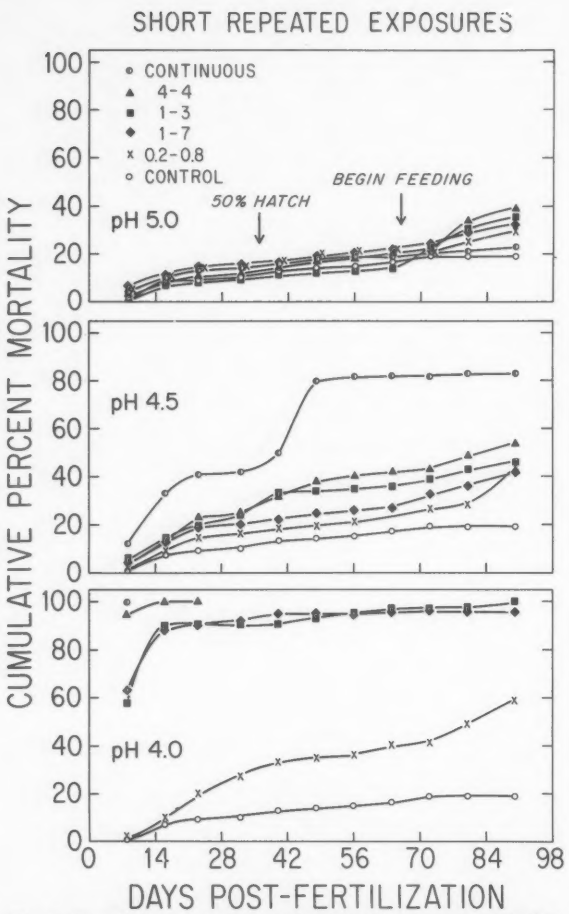

FIG. 1. Cumulative percent mortality of brook trout over their first 90 d of development when exposed to repeated, short-duration dilute sulfuric acid exposure episodes. Some characteristics of these regularly spaced, intermittent episodes (regimes) are described in Table 1. The embryonic stage preceded hatching (see arrows) and was characterized by presence of the chorionic membrane. Presence of a visible yolk sac in hatched animals defined the "alevin" stage. Shortly after initiation of feeding (see arrows), yolk sacs were completely absorbed defining the "fry" stage.

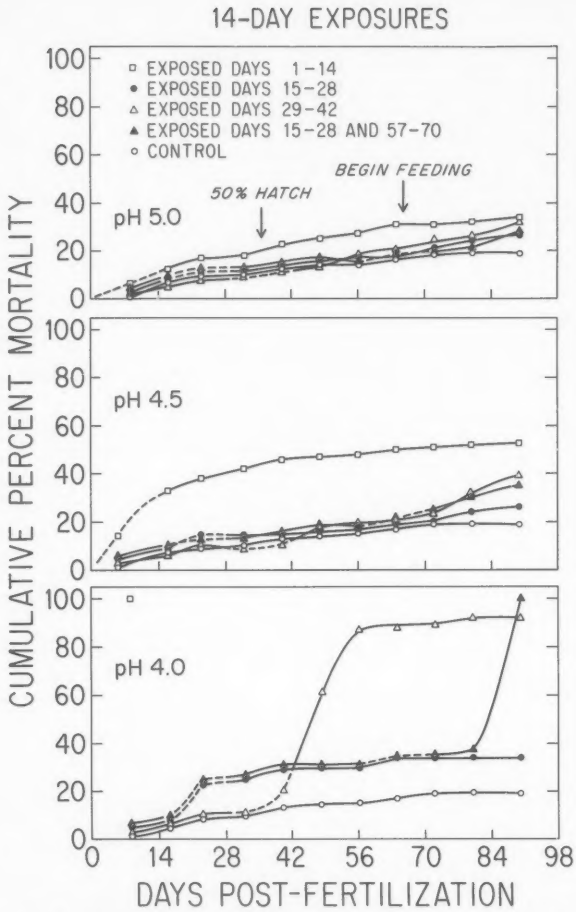

FIG. 2. Cumulative percent mortality of brook trout over their first 90 d of development when exposed to 14-d pulses of dilute sulfuric acid. Exposures were timed to coincide with major development stages. Broken segments of the lines indicate periods of H^+ exposure. Definitions of each stage are as in Fig. 1.

μmol H^+/L), and to 0 at pH 4.0 (100 μmol H^+/L). For repetitive, intermittent exposures the maximum [H^+] attained was the primary determinant of toxicity, secondarily modified by frequency and duration of the H^+ pulse.

At all pH's tested, lethality of repetitive intermittent exposures of 1 d was little mitigated by increasing the recovery period from 3 to 7 d (Fig. 1). Mortality of trout embryos exposed to repetitive H^+ pulses of pH 4.0 was about 90% after 3 wk of exposures for both these regimes. In the case of the 0.2–0.8 regime at pH 4.0 the relatively short exposure duration (4.5 h) and approximate 1-d recovery period (19.5 h) was considerably less lethal than other repetitive exposures (Fig. 1). Forty-one percent of the organisms survived daily 4.5-h pulses of pH 4.0 for 90 consecutive days. High mortality resulted at pH 4.0 in the 4–4 regime and began at the same time as in other short-duration regimes (Fig. 1). Ninety-five percent of the embryos died during the first 4-d pulse and none survived the second. Lethality of pH 4.5 and 5.0 pulses differed little between repetitive regimes (Fig. 1).

We simulated snowmelt event H^+ toxicity with sequential pulses at various times after fertilization. The effects of single 14-d H^+ pulses at pH 4.0, 4.5, or 5.0 differed greatly with developmental stage of brook trout (Fig. 2). A 14-d H^+ pulse immediately after fertilization (days 1–14), or over the hatching period (days 29–43), produced marked H^+-dependent mortality. A refractory embryonic period (days 15–28) was clearly evident. This latter regime was the least deleterious intermittent regime at all pH's tested although mortality at pH 4.0 was 34% compared with controls at 19%.

A decline in sensitivity of embryos to H^+ during the first 3 d after fertilization was demonstrated at pH 4.0 with the 4–4 regime. When the first 4-d H^+ pulse was begun on day 1, 2, or 3 after fertilization, there was 95, 23, and 7% mortality of the embryos in the respective groups within the first week of the test (a single 4-d H^+ pulse in each instance). After 16 d (two H^+ pulses), respective mortalities reached 100, 85, and 69%. Cumulative mortality progressively increased (1–6% per

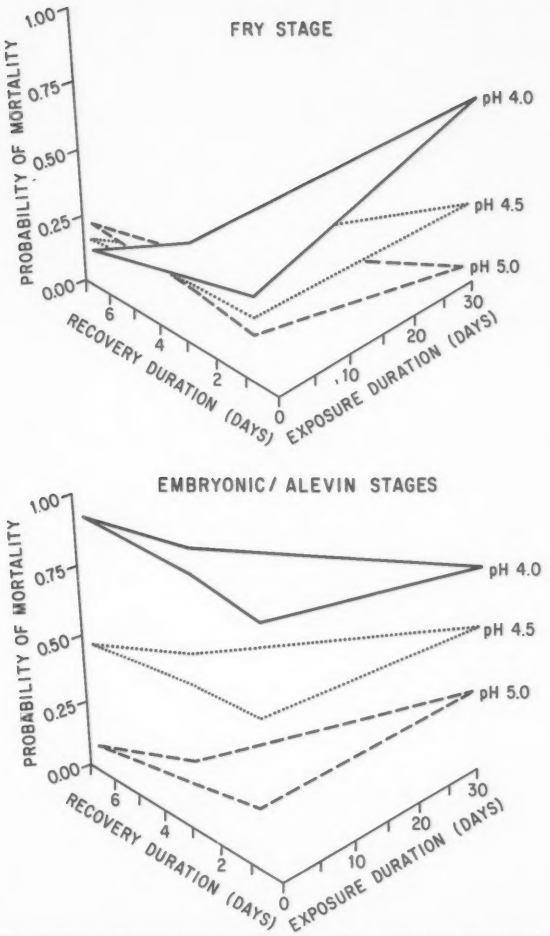

FIG. 3. Three-dimensional representation of an estimated relationship between mortality, recovery duration, and exposure duration at pH 4.0, 4.5, and 5.0 for early life stage brook trout before (upper panel) and after initiation of active swimming (lower panel). Response surfaces were generated as described in the text. We have only shown areas of predicted surfaces which are within the ranges of conditions tested (i.e. interpolated, not extrapolated areas).

exposure cycle) in both remaining groups thereafter (data not presented). Repetitive pH 4.0 pulses produced virtually complete mortality in all groups by 90 d (1% survived in the group begun on day 3).

Mortality during hatching was at least partially attributed to inability of embryos to successfully escape the chorion. The time to 90% hatching was delayed 1–8 d by H^+ exposure compared with the pH 6.8 control, except the 0.2–0.8 regime. The longest delays in hatching were 7 d at continuous pH 4.5 (continuous pH 4.0 was lethal) and 7 d at pH 4.0 for days 29–43 (hatching period). Earlier 2-wk exposures resulted in no more than 1 d of delay, while for the 1–3 and 1–7 regimes, delays of only 1–2 d resulted.

Relationships between exposure duration, length of recovery period, and mortality at our test pH's for repetitive dosing regimes were analyzed by response surface methods (Fig. 3). This permitted rigorous evaluation of contributions of various components of intermittent exposure and yielded reasonably good correspondence between observed and predicted mortality (Table 3). Responses to exposure duration at a given pH were ameliorated less by increased length of recovery period than simple proportions (Table 1) would predict. Statistical analysis of the model's empirical parameters indicated that data for embryos and alevins were described by one exposure–response relationship and data for fry by another (Fig. 3). Simple cumulative mortality also indicated that H^+ sensitivity changed with developmental stage (Fig. 1). After hatching, marked changes in exposure–response relationships occurred, probably due to removal of the buffering effects of the chorion. A basis for sensitivity change at onset of feeding is discussed below. The forms of response surfaces generated by our model for the fry stage showed the predominant role of exposure duration as a determinant of H^+ toxicity (Fig. 3, upper panel).

Yolk-free dry weight of brook trout was first determined 2 wk after hatching. No $[H^+]$-dependent effect on weight was apparent in any group surviving to this time (Table 4). At 28 d after hatching, significant weight reduction (37%) was found only in fish continuously exposed to pH 4.5. Insufficient fish exposed to continuous pH 4.0 survived to permit sampling. No significant effects on dry weight were detected at 42 d. Significant reductions in dry weight of fish surviving 90 d for continuous exposure to pH 4.5 (34%), the 1–7 intermittent regimen at pH 4.0 (15%), and the 4–4 intermittent exposure at pH 4.5 (41%) were found.

Changes in Na^+, K^+, and Ca^{2+}

We measured whole-fish Na^+, K^+, and Ca^{2+} concentrations at two times in the embryo/larval experiment to assess sublethal effects attributable to H^+ action at the primary target organ, the gill (McDonald 1983). Higher electrolyte concentrations of fish at 60 as compared with 90 d of development were explained by the presence of yolk at the earlier time point (Fig. 4). To facilitate comparisons of regime-dependent differences in sublethal H^+ effects, means of $[H^+]$ over the entire exposure period were calculated (Fig. 4). Exponential relationships between $[H^+]$ and decreased Na^+ and Ca^{2+} contents were apparent after 60 d in trout surviving continuously (Fig. 4, open symbols) but not cyclically intermittent exposures. Significantly decreased K^+ content occurred only in fish given a daily 4.5-h pulse of pH 4.0 water; all other pH 4.0 exposed fish were dead. After 90 d of exposure, $[H^+]$-dependent ionoregulatory dysfunction was apparent in whole-body Na^+, K^+, and Ca^{2+} contents (Fig. 4, solid symbols). Analysis of variance also indicated that after 90 d, all repetitive intermittent exposure regimes except 0.2–0.8 significantly decreased whole-body K^+ and Ca^{2+} contents. Whole-body, Na^+ was only decreased by the 4–4 and 1–3 intermittent regimes. Mean $[H^+]$-dependence was well modelled ($r^2 > 0.75$ in all cases) by an exponential relationship, but each regime fitted a separate curve.

Discussion

A threshold exposure duration for H^+ toxicity was apparent in acute toxicity tests with rainbow trout and after weeks of episodic exposure of brook trout. This indicated consistency of our results between salmonid species. However, when H^+ pulses as brief as 4.5 h/d were continued for up to 90 d, this threshold was no longer discernible in our embryo/larval study. This supported the importance of long-term experiments in

TABLE 3. Correspondence between observed mortalities and those predicted by a response surface model for intermittent exposure of early life stages of brook trout to dilute sulfuric acid.

		Embryos/alevins[a]		Fry[b]	
pH	Treatment regime	Observed mortality (%)	Predicted mortality (%)	Observed mortality (%)	Predicted mortality (%)
6.8	Continuous	17	8	2	0
5.5	Continuous	15	25	5	2
5.0	Continuous	18	24	4	9
	4–4	18	14	22	22
	1–3	14	14	24	18
	1–7	20	6	15	21
	0.2–0.8	20	20	11	16
4.5	Continuous	82	61	13	34
	4–4	78	83	33	23
	1–3	33	49	14	19
	1–7	33	46	20	15
	0.2–0.8	22	51	25	22
4.0	Continuous	100	79	84	72
	4–4	96	89	72	33
	1–3	96	85	50	50
	1–7	95	92	17	17
	0.2–0.8	41	82	31	29

[a]Percentages reflect cumulative mortalities either observed or predicted over the first 62 d of the 90-d experiment for those animals exposed to repetitive pulses of H^+. Each group began with between 426 and 461 embryos.

[b]Percentages reflect cumulative mortalities either observed or predicted over the final 27 d of the study. Each exposure group began with 100 fry except in those groups where previous mortalities limited sample size: 4–4 at pH 4 ($n = 18$), 1–3 at pH 4 ($n = 2$), 1–7 at pH 4 ($n = 6$), continuous pH 4.5 ($n = 56$), and 0.2–0.8 at pH 4.5 ($n = 67$).

assessing exposure condition modulated toxicity. Further, little if any amelioration of toxicity was achieved when recovery period was increased from 3 to 7 d between 1-d H^+ pulses over a 90-d experiment. Repetitive episodic H^+ exposures appeared to be more toxic to fish than would be predicted from cumulative response to mean $[H^+]$ over time.

Exposure regimes for H^+ were designed to mimic short-term episodes associated with acidic rainstorm events and longer term snowmelt-type situations. The highly structured exposure patterns used in this study can not be directly extrapolated to stochastic acidic precipitation events but do demonstrate the importance of understanding the roles of time–concentration relationships in H^+ exposure. In a more general sense, these experiments provided data for systematic analysis of contributions of various components of intermittent exposure to chemical toxicity. Acute lethality of intermittent exposures suggested that at equivalent mean $[H^+]$, toxicity increased with exposure duration and/or decreased with length of recovery period (Table 2). Results from our embryo/larval experiment indicated more complex relationships after repetitive exposures for 90 d. Cumulative mortality in regimes with lower proportions of H^+ exposure per cycle (0.2–0.8, 1–7) increased to values similar to regimes with higher proportions of H^+ exposure per cycle (1–3, 4–4) by the end of the embryo/larval test (Fig. 1). Only at pH 4.0 could this be explained by convergence of responses in all regimes towards 100% mortality. Regime-dependent differences in mortality, growth, and ionic composition were more clear when time course data were studied. Expressing exposure data in terms of mean $[H^+]$ integrated the time–concentration curves and permitted more direct comparison of regimes (Fig. 4; Table 4). This approach has both advantages and limitations. For example, when comparing reduced Na^+, K^+, and Ca^{2+} contents of fish at the end of the test, depressed tissue concentrations were more marked after three of four intermittent exposure regimes (4–4, 1–3, 1–7) than after continuous exposures to equivalent mean $[H^+]$ (Fig. 4). However, when calculating mean $[H^+]$, all information concerning temporal aspects of exposures (i.e. duration, recovery period) was lost.

Response surface methods were used to visualize relationships between percent mortality, pH, exposure duration, and recovery duration. Although the true relationship among these variables was unknown, it was approximated reasonably well by a polynomial through use of the logistic model. This model was only applicable within the range of conditions used in the experiment (Fig. 3) but allowed simultaneous evaluation of key parameters and identified exposure duration as a predominant determinant of toxicity for repetitive, intermittent exposures to H^+. This generalization was also apparent, but not readily amenable to statistical analysis, when time course data for cumulative mortality were examined (Fig. 1).

Analysis of the response surface model (Fig. 3; Table 3) provided insight into toxicity of repetitive pulses of H^+ to early life stages of brook trout. Two nonlinear relationships between the ratio of exposure duration to recovery period were fitted by this statistical model. One applied throughout the embryonic and alevin stages and the other to fry. A biological basis for this appears most well founded in fry exposed to pH 4.0 and 4.5 pulses. Organisms that survived the first 60 d of the study represented H^+-resistant populations (except controls). The extent of resistance, however, likely varied with exposure regime. Short-duration H^+ exposures with relatively long

TABLE 4. York-free dry weight (mg) of brook trout alevins and fry at various times during 90 d of continuous or repetitive intermittent exposures to dilute sulfuric acid. Results are means ± SE for 10 individual fish, except where otherwise indicated. NA = insufficient animals were available for weight determination at this time due to high mortality in this group. *Individual weight was significantly different at $p < 0.05$ from value for fish continuously exposed at pH 6.8 at this time. Methods for statistical analysis are presented in the text.

Days of study	Regime (days at reduced pH – days at control pH)															
	0.2 – 0.8			1 – 7			1 – 3			4 – 4			Continuous			
	4.0	4.5	5.0	4.0	4.5	5.0	4.0	4.5	5.0	4.0	4.5	5.0	4.0	4.5	5.0	5.5	6.8
48	3.0±0.2	3.2±0.2	3.1±0.1	2.9±0.2	2.9±0.2	2.9±0.2	2.4±0.1	2.7±0.1	3.2±0.2	2.5±0.2	2.4±0.2	2.8±0.2	NA	2.3±0.1	2.9±0.2	2.7±0.1	3.0±0.1
62	6.3±0.4	7.3±0.2	6.6±0.3	5.9±0.3	6.6±0.3	6.7±0.3	5.8±0.4	6.3±0.3	6.4±0.4	5.4±0.4	5.8±0.4	5.3±0.4	NA	4.6±0.3*	6.6±0.4	6.7±0.4	7.3±0.2
76	15.4±0.9	14.6±1.0	15.4±1.1	NA	15.2±0.7	17.3±0.3	11.6[a]	14.6±1.0	15.9±0.8	NA	13.9±0.8	16.0±1.2	13.4±1.1	13.5±1.0	15.7±1.0	16.5±1.8	15.4±1.0
90	17.6±1.2	17.4±1.6	19.0±1.5	14.7±2.4*	16.3±1.7	16.7±2.3	10.7[a]	19.8±1.4	17.1±1.2	10.2±0.5[b]*	16.0±1.5	17.1±1.2	NA	11.4±0.5*	17.2±1.6	18.1±1.3	17.2±0.8

[a]Only a single fish was available for weight determination.
[b]Only four individuals were available for weight determination.

FIG. 4. Influence of continuous or cyclic intermittent H^+ exposures on whole-body electrolyte content of brook trout 60 d (open circles and ×'s) or 90 d (solid symbols) after fertilization. H^+ concentration was expressed as the mean over the length of the experimental period prior to sampling time. Only those exposure groups which exhibited a significant dose effect, as indicated by one-way ANOVA, were plotted. Results are means ± SEM for four fish in each regime. An exponential function modeled the dose–response relationships for the continuous exposure regime.

recovery periods probably exerted less rigorous selective pressure (particularly 0.2–0.8). Ionoregulatory dysfunction secondary to impaired ion regulation by the gill was the predominant mode of H^+ toxicity in freshwater fish exposed to pH and water quality conditions relevant to this study (McDonald 1983). Increased oxygen demand coincident with active swimming and feeding after 60 d of development required increased ventilation of the gills. Toxicity of regimes which produced marginal responses in embryos/alevins was exacerbated in the more active fry. Groups that were not strongly selected for H^+ resis-

tance (0.2–0.8 at pH 4.5 and 4.0) experienced marked mortality late in the study (Fig. 1).

Our results (Fig. 2) confirm earlier reports on differing sensitivities of developmental stages of salmonid embryos to H^+ (reviewed by Haines 1981). Embryos exposed to pH 4.0 and 4.5 during days 1–14 after fertilization exhibited high mortality. Little mortality occurred in groups exposed during days 15–28. This 14-d exposure occurred entirely between the sensitive early cleavage stages and the onset of increased sensitivity at hatching (Menendez 1976). Sharply increased mortality occurred during hatching of fish continuously exposed to pH 4.5 (Fig. 1) and those pulsed with pH 4.0 during days 29–42 (Fig. 2). This was also observed for H^+-exposed Atlantic salmon (*Salmo salar*) and was attributed to chorionase inhibition (Daye and Garside 1980; Peterson et al. 1980). The shortest exposure duration at pH 4.0 produced mortality only slightly higher than controls until after hatching (Fig. 1). The duration of this exposure (0.2–0.8) was probably too brief for the H^+ gradient to equilibrate across the chorion (Daye and Garside 1980). Atlantic salmon embryos exposed to H^+ pulses throughout development were not delayed from hatching, and acclimation of early stages was concluded (Peterson et al. 1980). This phenomenon was not observed in our brook trout experiments.

Sensitivity to H^+ during early embryonic cleavage and hatching was anticipated. Reduced survival for intermittent regimes with a peak $[H^+]$ at pH 5.0 in comparison with continuous exposure at that level was surprising (Fig. 1). This suggested that moderate H^+ pulses associated with acidic rainstorm (events influencing small streams) could be more deleterious than would be indicated by continuous exposure experiments. Survival after intermittent pH 4.5 and 4.0 pulses was greater than for continuous exposures to these levels. This was intuitive and consistent with our previous findings with other toxicants (Seim et al. 1984; Curtis et al. 1985; Siddens et al. 1986).

Recovery periods mitigated or had little effect on toxicity of intermittent chemical (Gardner et al. 1977; Coffin et al. 1977; Ingersoll and Winner 1982) and radiation (Burns and Albert 1978; Burns et al. 1978) exposures. In our experience with 90 d of repetitive chemical exposures, toxicity and accumulation of copper and fenvalerate were approximately doubled in fish exposed to daily toxicant pulses as compared with continuous exposures at equivalent mean concentrations (Seim et al. 1984; Curtis et al. 1985). An exception to this generality occurred at relatively low peak intermittent concentrations when elimination processes were probably not saturated throughout the recovery period. One goal of this research was to identify components of time–concentration relationships which explain such differences. In the case of H^+, recovery period and percent time exposed were less important than anticipated. Exposure duration and peak $[H^+]$ appeared to be the primary determinants for toxicity of episodic reductions in PH to trout. The exposure duration which resulted in significant toxicity decreased with the number of exposure episodes.

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Groundwater–Surface Water Relationships in Boreal Forest Watersheds: Dissolved Organic Carbon and Inorganic Nutrient Dynamics[1]

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Ford, T. E., and R. J. Naïman. 1989. Groundwater–surface water relationships in boreal forest watersheds: dissolved organic carbon and inorganic nutrient dynamics. Can. J. Fish. Aquat. Sci. 46: 41–49.

Dissolved organic carbon (DOC) and inorganic nutrients (NH_4-N, NO_3-N, soluble total N, PO_4-P, soluble total P, and Si) were measured in ground and surface waters in the Matamek River drainage network, Quebec, Canada. In general, concentrations of carbon and nitrogen were significantly higher in groundwater than in surface water (up to 340% for DOC and up to 700% for total N). No significant difference was detected for phosphorus whereas considerable variation occurred for silicon, with significantly higher groundwater concentrations at 50% of the study sites. We hypothesize that (1) groundwater is a source of DOC and nitrogen in these systems and (2) nutrients introduced through groundwater seepage are rapidly utilized via oxidative, biotic processes within the hyporheal zone or at the sediment–water interface.

On a mesuré les concentrations de carbone organique dissous (COD) et de substances nutritives inorganiques (NH_4-N, NO_3-N, N soluble total, PO_4-P, P soluble total et Si) dans les eaux souterraines et de surface du réseau hydrographique de la rivière Matamek, au Québec (Canada). En règle générale, les concentrations de carbone et d'azote étaient notablement plus élevées dans les eaux souterraines que dans les eaux de surface (dans les eaux souterraines, les concentrations de COD atteignaient jusqu'à 340 % des valeurs mesurées dans les eaux de surface et, pour les concentrations de N total, ce pourcentage atteignait jusqu'à 700 %). Les écarts entre les teneurs en phosphore étaient négligeables, tandis que les concentrations de silicium variaient considérablement : dans 50 % des zones d'étude, les concentrations de silicium étaient notablement plus élevées dans les eaux souterraines. Nous avançons donc les hypothèses suivantes : (1) les eaux souterraines constituent une source de COD et d'azote dans ces réseaux, et (2) les substances nutritives introduites par infiltration des eaux souterraines sont rapidement utilisées via des processus oxydatifs et biotiques dans la zone hyporthéique ou à l'interface sédiment–eau.

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Most dissolved material from the atmosphere and from terrestrial plants percolates downward through the soil where partial mineralization by soil microbial communities occurs (Likens et al. 1977; Wallis et al. 1981). A proportion of the dissolved material, presumably rich in microbially recalcitrant compounds, eventually reaches the groundwater. Groundwater is considered the major component of flow for small streams throughout the year (Freeze 1974; Viessman et al. 1977).

Until recently, groundwater was thought to neither dilute nor concentrate the organic carbon pool in streams and rivers and to consist mainly of refractory, large molecular weight molecules (de Haan 1972; Otsuki and Wetzel 1973; McDowell and Fisher 1976; Larson 1978). Wallis (1981) and Naiman et al. (1987), however, presented evidence that dissolved organic carbon (DOC) from groundwater can contribute significantly to the carbon budget of streams and rivers and hypothesized that groundwater may contain large amounts of labile organic matter. Groundwater also may be a major source of nitrogen to surface water in the form of ammonium; however, phosphorus levels are generally considered to be low due to the insoluble nature of phosphate-containing minerals (Wetzel 1983). Groundwater may also be an important source of silicon, stimulating diatom production in surface waters (Hurley et al. 1985).

These observations, in combination with the inability of Naiman et al. (1987) to explain discrepancies between measured input and output rates of organic carbon and inorganic nitrogen for streams in the Matemek and Moisie River watersheds, Quebec, led to an investigation of processes occurring at locations with active groundwater discharge. The objective was to investigate relationships between groundwater and surface water DOC and nutrient concentrations in boreal forest headwater streams. Pristine conditions exist in these streams; hence, carbon and nutrient fluxes could be studied without the complication of industrial or domestic contamination.

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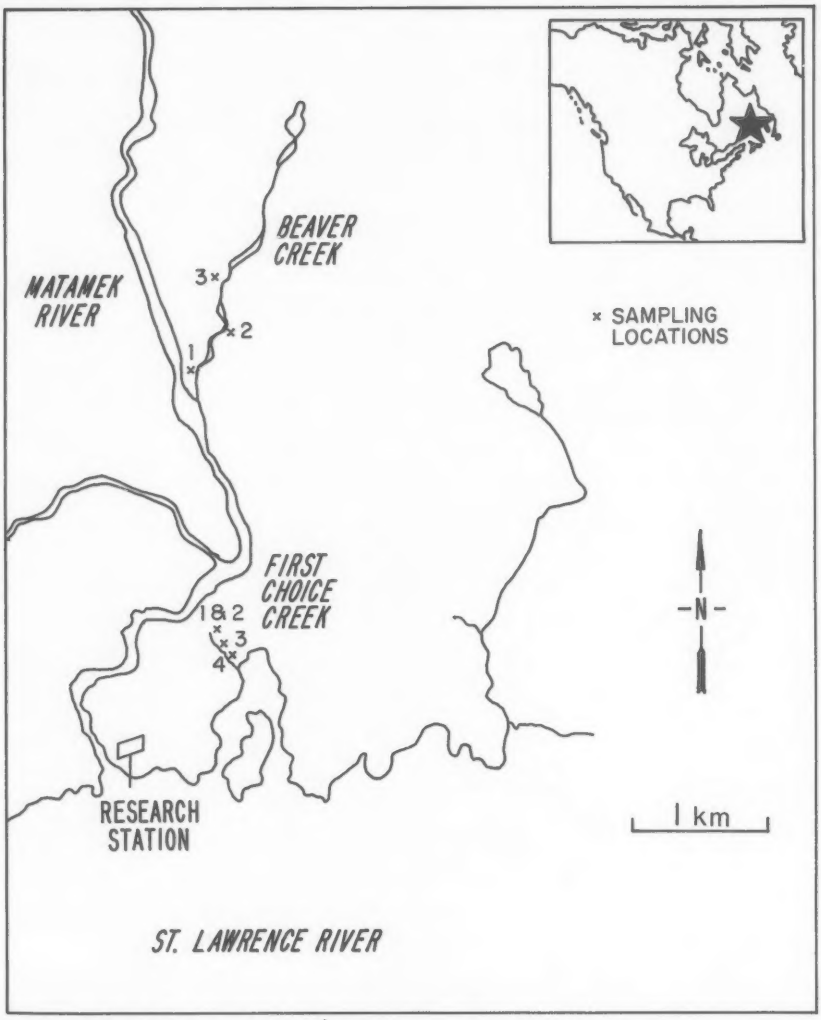

FIG. 1. Location of study areas near Sept Îles, Quebec. Sites for groundwater and surface water sampling are indicated for First Choice Creek and Beaver Creek.

TABLE 1. Methods used in water chemistry analysis.

Analysate	Method	Reference	Sensitivity (mg C, N, P, or Si/L)
DOC	UV oxidation	Naiman 1982	0.05
NH_4	Phenolypochlorite	Solorzono 1969	0.002
NO_3	Cadmium–copper reduction	Wood et al. 1967	0.0015
SN	Persulfate digestion	D'Elia et al. 1977	0.030
PO_4	Single solution	Murphy and Riley 1962	0.001
SP	Persulfate oxidation	Menzel and Corwin 1965	0.001
Silicates	Colorimetric technique	Strickland and Parsons 1968	0.008

Materials and Methods

Study Sites

Two streams in the boreal forest near Sept Îles, Quebec, Canada, were chosen (Fig. 1). Both streams have been previously described (Naiman 1982, 1983a, 1983b). First Choice Creek is a first-order, spring-fed stream and differs from other streams in the area in that the water is clear. Four sites were chosen on First Choice Creek, two at the spring fed source and two 25 and 50 m downstream. Beaver Creek (second-order) has the characteristic humic-coloured water of this region and

consists of a series of beaver ponds separated by short riffles. Three sites were selected along Beaver Creek; all were situated at small seeps draining into the creek. Sampling sites were chosen to represent the range of environments encountered in each stream. The landscape is typical of the subarctic Precambrian Shield with numerous muskegs, podzolic soils containing pale sandy soil beneath layers of slowly decaying litter and humus, and terrestrial vegetation consisting of mostly black and white spruce (*Picea glauca* and *P. mariana*) and balsam fir (*Abies balsamea*).

Sample Collection

Lysimeters were set 50 cm into the sediment, one at each sample site shown in Fig. 1. Lysimeters were placed in position in May 1983, 1 yr before actual sampling began to ensure minimal contamination. Samples were taken weekly during the ice-free season of May-October 1984. Prior to initial sampling, the interior of each lysimeter was rinsed several times in situ with ultrapure water (Millipore "milli-Q" system); the first water collection was then discarded. Groundwater was collected over a 24-h period with an initial 150 cm of vacuum pressure in the lysimeter. If less than 250 mL was collected, then a further sample was taken over the next 24 h and pooled with the first sample. Samples of surface water from the nearest source (1–5 m) of running water to each lysimeter were also collected for carbon analysis as above. Nutrients in surface water were measured in First Choice Creek 50 m from the source and from two sites from Beaver Creek (riffle section between lysimeter sites 2 and 3 and a beaver pond between lysimeter sites 1 and 2; see Fig. 1).

The water was filtered through a preashed Gelman AE glass fiber filter (nominal pore size 0.5 μm). A subsample of 50 mL of filtrate was analyzed for DOC on a Dohrman model 54 organic carbon analyser; the remainder was frozen for subsequent nutrient analysis. Nutrients measured were ammonium (NH_4-N), nitrate (NO_3-N), soluble total nitrogen (SN), orthophosphate (PO_4-P), soluble total phosphorus (SP), and silicon (method references and detection limits in Table 1). Nutrient concentrations are affected by time between field collection, filtration, and freezing. This was minimized where possible. However, some lag time (<3 h) was inevitable and may contribute variability to the inorganic nutrient data.

Statistical Analysis

Site-specific groundwater and surface water DOC and nutrient concentrations were compared using the paired-sample *t*-test (Zar 1984). Groundwater and surface water concentrations between stream catchments were compared by applying the paired-sample *t*-test to mean concentrations per sampling period. Linear regression analysis was used to test for downstream trends in the First Choice Creek catchment (Zar 1984).

Results

Dissolved Organic Carbon

Mean DOC concentrations (Table 2) were low for all surface water samples and for the three groundwater sample sites nearest the stream source in First Choice Creek. However, the furthest groundwater site (seep 4) had higher concentrations of DOC. There was a progressive increase in both groundwater and surface water DOC concentrations from the stream source to the furthest site ($r^2 = 0.99$ and 0.97, respectively). Seeps 1 and 2 showed lower DOC concentrations in groundwater than in surface waters ($t = 3.2$, $p < 0.01$ and $t = 4.5$, $p < 0.001$, respectively) whereas both seeps 3 and 4 showed higher concentrations of DOC in groundwater than surface water ($t = 2.6$, $p < 0.01$ and $t = 8.3$, $p < 0.001$, respectively). Peaks in DOC in July (Fig. 2) were probably due to the concentrating effects of seasonally low discharge.

Beaver Creek had 10-fold higher concentrations of surface water DOC than First Choice Creek (Table 2). Groundwater seeps 1 and 3 had concentrations of DOC similar to surface water ($t = 1.0$, NS and $t = 0.8$, NS, respectively) but seep 2 had higher concentrations ($t = 6.5$, $p < 0.001$) than surface water until the end of the summer. Unlike First Choice Creek, no obvious peak in DOC levels occurred in July (Fig. 2), despite low discharge.

Nitrogen

Soluble total nitrogen in First Choice Creek groundwater at seeps 3 and 4 (Table 3) was substantially higher than concen-

TABLE 2. Mean DOC concentrations in mg C/L (± SD) for groundwater and surface water samples from First Choice Creek and Beaver Creek catchments sampled weekly from May to October 1984 ($n = 19$).

Site	Groundwater	Surface water	Ratio (groundwater to surface water)
First Choice Creek			
Seep 1	0.96	1.13	0.85
	(0.39)	(0.39)	
Seep 2	0.86	1.15	0.75
	(0.41)	(0.54)	
Seep 3	1.56	1.34	1.16
	(0.35)	(0.49)	
Seep 4	6.42	1.89	3.40
	(2.48)	(0.49)	
Beaver Creek			
Seep 1	10.81	10.15	1.07
	(2.16)	(3.18)	
Seep 2	25.43	10.27	2.48
	(7.67)	(3.56)	
Seep 3	11.40	11.97	0.95
	(1.93)	(3.26)	

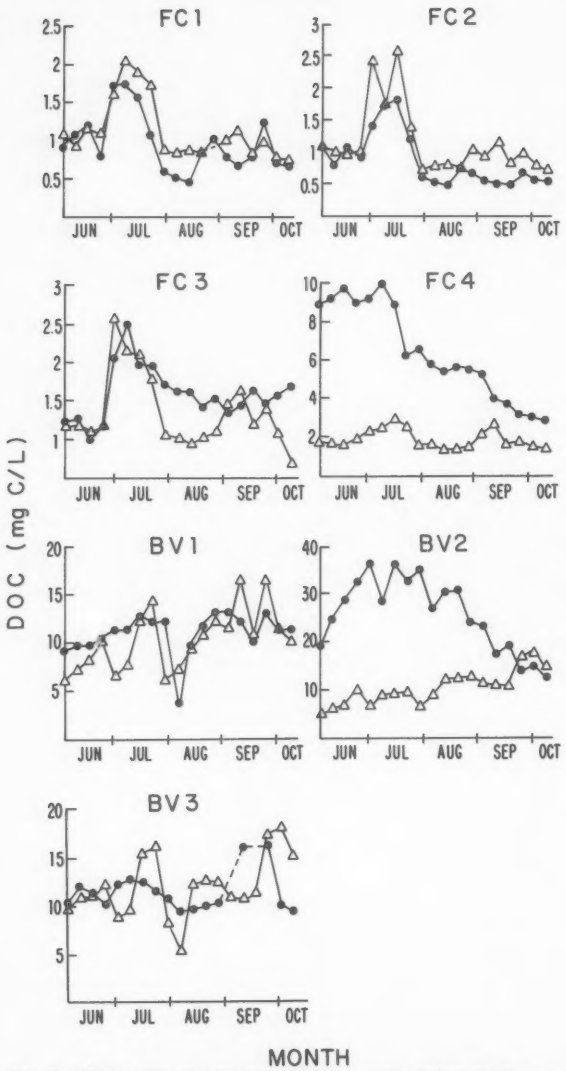

FIG. 2. DOC concentrations for groundwater and surface water samples taken weekly from First Choice Creek (FC) and Beaver Creek (BV) catchments from May to October 1984. Groundwater = solid circles, and surface water = open triangles. Note that the scales for DOC concentration differ between sampling sites.

trations in surface water ($t = 8.0$, $p < 0.001$ and $t = 7.0$, $p < 0.001$, respectively), but only slightly higher than in surface water at seeps 1 and 2 ($t = 2.9$, $p < 0.01$ and $t = 3.9$, $p < 0.001$, respectively). Ammonium predominated in groundwater at seeps 3 and 4 and nitrate predominated at seeps 1 and 2 and in surface water, suggesting oxidizing conditions at seeps 1 and 2. Nitrite (NO_2-N) concentrations were below the detection limit of the analysis (<0.001 mg N/L) in both groundwater and surface water samples. Seasonal variation in nitrogen levels (Fig. 3a–3c) show considerable variation in soluble total nitrogen at seeps 3 and 4, reflecting peaks in ammonium, particularly at seep 4 during June and early July. Highest nitrate concentrations were obtained in June in surface water and at seeps 1 and 2 whereas at seep 4, nitrate concentrations increased gradually over the summer.

Mean groundwater and surface water nitrogen concentrations were significantly higher in the Beaver Creek catchment than in the First Choice Creek catchment (Table 3; for pooled data, $t = 16.6$, $p < 0.001$ and $t = 11.8$, $p < 0.001$, respectively). Groundwater samples in Beaver Creek had far higher nitrogen concentrations than the surface water, particularly at seep 2 (112-fold higher ammonium, Table 3). Nitrogen was predominantly in the form of ammonium at seeps 1 and 2, but at seep 3, ammonium concentrations were similar to surface water concentrations and only 1.5-fold higher than seep 3 nitrate concentrations. As at First Choice Creek, nitrite concentrations were all extremely low at Beaver Creek (<0.001 mg N/L). Seasonal variation in nitrogen concentrations (Fig. 3d–3f) was greatest in groundwater at seeps 1 and 2. However, there is no apparent correlation between the sites with peaks in July and September at seep 1 and high concentrations from July through September at seep 2. Variation in soluble total nitrogen concentrations was also reflected in ammonium concentration at seep 2 and to a lesser extent in the other waters. Pond and riffle waters had almost identical concentrations of nitrate ($t = 0.16$, NS) with highest concentrations through August and the beginning of September. Groundwaters had generally lower concentrations of nitrate than pool or riffle waters (for pooled data, $t = 2.9$, $p < 0.01$); however, seep 2 concentrations peaked at the end of August and continued high to the end of September.

Mean carbon to nitrogen ratios (C:N) were significantly higher in surface water than in groundwater. The mean surface water C:N ratio was 55 for First Choice Creek but was 17 for groundwater ($t = 3.4$, $p < 0.005$). The difference was also striking in the Beaver Creek catchment with a surface water C:N ratio of 44 and a groundwater C:N ratio of 18 ($t = 10.5$, $p < 0.001$). Ratios for the groundwater reflect the high concentrations of nitrogen.

Phosphorus

Soluble total phosphorus concentrations were low (mean <0.006 mg P/L) in the First Choice Creek catchment with slightly higher concentrations in surface water than in groundwater (Table 3, $t = 4.8$, $p < 0.001$). The ratio of orthophosphate to soluble total phosphorus was high in surface water (0.78) and water from spring seeps 1 and 2 (0.56 and 0.90, respectively). From seeps 3 and 4, however, the ratio was lower (<0.36 and 0.46, respectively). Reduced forms of phosphorus might be expected to predominate at these sites if low oxygen conditions are present, as the nitrogen data suggest. There were little seasonal variations in phosphorus concentrations and hence they are not shown. Coefficients of variation (CV) are given only where measurements did not fall below the detection limit of the method (0.001 mg P/L). Concentrations in surface water ranged from 0.003 to 0.007 mg P/L for orthophosphate (CV = 22%) and from 0.003 to 0.008 mg P/L for total soluble phosphorus (CV = 23%). In groundwater, phosphorus concentrations ranged from below detection to 0.008 mg P/L for orthophosphate and from 0.001 to 0.010 mg P/L for soluble total phosphorus (CV for pooled data = 34%).

Soluble total phosphorus concentrations were also low in Beaver Creek (mean <0.008 mg P/L), with considerably higher concentrations in groundwater than in surface water ($t = 2.9$, $p < 0.01$). The ratio of orthophosphate to soluble total phosphorus was low in both surface water and groundwater (surface

TABLE 3A. Inorganic nutrient concentration in surface and subsurface water samples from two stream catchments in Quebec. Concentrations of nutrients are in mg/L and are mean values (± SD) for samples collected weekly from May to October, 1984. (n = 19, unless otherwise indicated).

Site	NH_4	NO_3	SN	PO_4	SP	Si
First Choice Creek						
Surface water	0.0045	0.0109	0.0454	0.0046	0.0059	1.0802
	(0.0029)	(0.0134)	(0.0313)	(0.0011)	(0.0014)	(0.6271)
Seep 1	0.0074	0.0128	0.1018	0.0023	0.0041	0.4502
	(0.0032)	(0.0152)	(0.0566)	(0.0036)	(0.0039)	(0.1953)
Seep 2	0.0093	0.0243	0.0676	0.0026	0.0029	0.5619
	(0.0037)	(0.0289)	(0.0332)	(0.0035)	(0.0013)	(0.2992)
Seep 3	0.0971	0.0038	0.1688	<0.001	0.0028	2.7167
	(0.0224)	(0.0026)	(0.0487)	—	(0.0013)	(1.2593)
Seep 4	0.0914	0.0149	0.2417	0.0018	0.0039	1.0655
	(0.0771)	(0.0088)	(0.1198)	(0.0016)	(0.0019)	(0.7866)
Beaver Creek						
Surface water pond	0.0094	0.0326	0.2382	0.0011	0.0033	0.2751
	(0.0082)	(0.0244)	(0.0662)	(0.0008)	(0.0007)	(0.2193)
Riffle	0.0163	0.0311	0.2601	0.0013	0.0038	0.3179
(n = 18)	(0.0130)	(0.0255)	(0.0781)	(0.0008)	(0.0010)	(0.2755)
Seep 1	0.2355	0.0057	0.4801	0.0014	0.0043	1.4996
	(0.0340)	(0.0027)	(0.1536)	(0.0010)	(0.0014)	(0.7816)
Seep 2	1.4353	0.0309	1.8040	<0.001	0.0056	1.6954[a]
(n = 18)	(0.3403)	(0.0388)	(0.2574)	—	(0.0032)	(0.5881)
Seep 3	0.0174	0.0117	0.2800	0.0034	0.0079	0.2331
(n = 16)	(0.0261)	(0.0130)	(0.0696)	(0.0072)	(0.0088)	(0.2506)

TABLE 3B. Ratio of groundwater to surface water nutrient concentrations (mean of pond + riffle for Beaver Creek).

First Choice Creek						
Seep 1	1.6	1.2	2.2	0.5	0.7	0.4
Seep 2	2.1	2.2	1.5	0.6	0.5	0.5
Seep 3	21.6	0.3	3.7	<0.2	0.5	2.5
Seep 4	20.3	1.4	5.3	0.4	0.7	1.0
Beaver Creek						
Seep 1	18.3	0.2	1.9	1.2	1.2	5.1
Seep 2	111.7	1.0	7.2	<0.8	1.6	3.2
Seep 3	1.4	0.4	1.1	2.8	2.2	0.8

[a] n = 10.

water: 0.33; seeps 1–3: 0.33, <0.18, and 0.43, respectively). Surface water phosphorus concentrations ranged from below detection to 0.004 mg P/L for orthophosphate and from 0.002 to 0.007 mg P/L for soluble total phosphorus (CV = 20%). Groundwater phosphorus levels ranged from below detection to 0.004 mg P/L for orthophosphate and below detection to 0.013 mg P/L for soluble total phosphorus.

Silicon

Groundwater silicon concentration was highest in First Choice Creek at seep 3 (250% of the surface water concentration, $t = 5.9$, $p < 0.001$) and lowest at seeps 1 and 2 (48% of the surface water concentration, $t = 4.0$, $p < 0.001$, for both). Mean silicon concentration at seep 4 was the same as for surface water ($t = 0.06$, NS), but when seasonal variation was examined, a peak in silicon occurred at seep 4 in mid-July whereas surface water silicon concentration peaked in mid-September (Fig. 4a). Silicon concentrations at both seeps 3 and 4 were high in early to mid-July and there were smaller peaks during July in surface water and at seep 2. The largest concentration of groundwater silicon occurred at seep 3 in early September, corresponding to the highest concentration in surface water.

Surface water silicon concentrations were considerably lower in Beaver Creek than in First Choice Creek (27% of First Choice Creek surface water, $t = 6.8$, $p < 0.001$). Highest concentrations were found at seeps 1 and 2 (540% surface water concentration, $t = 7.6$, $p < 0.001$ and $t = 8.6$, $p < 0.001$, respectively) and lowest at seep 3 (79% surface water concentration, $t = 0.03$, NS). Seasonal variations (Fig. 4b) show peaks in silicon concentration at seep 2 in mid-July and at seep 3 in early August. Concentrations remained high at seep 1 during August and September after increasing throughout the season. Concentrations increased steadily in pond and riffle surface waters, peaking in early to mid-September and then decreasing rapidly.

Discussion

Concentrations of DOC in groundwaters were significantly higher than surface water concentrations at two sites and, there-

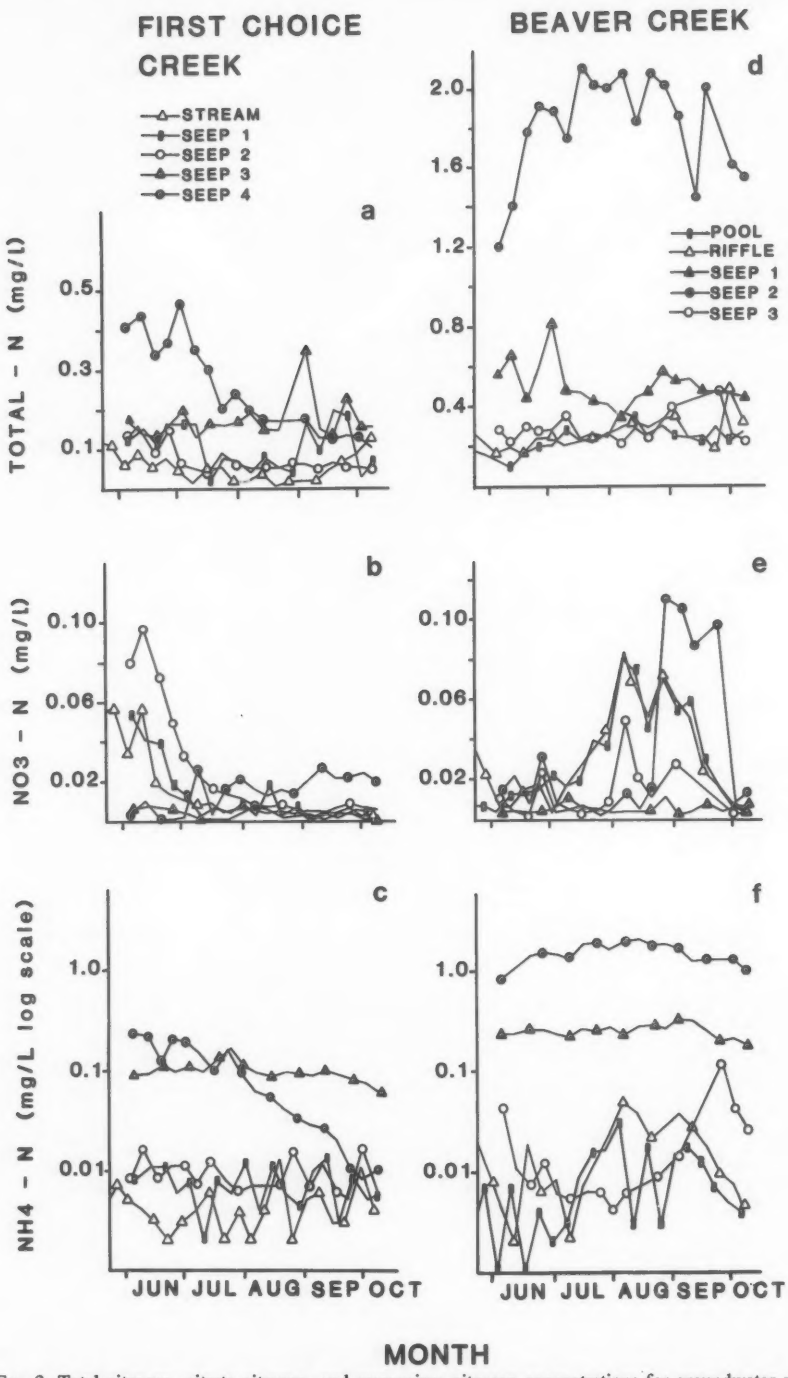

FIG. 3. Total nitrogen, nitrate nitrogen, and ammonium nitrogen concentrations for groundwater and surface water samples taken weekly from First Choice Creek and Beaver Creek catchments from May to October 1984.

fore, a potential source of DOC to the surface water. However, there is a large variation in the groundwater carbon inputs along stream systems. Surface water at the downstream First Choice Creek site had twice the DOC concentration of the surface water at the source, suggesting that either the groundwater was supplying DOC or considerable leaching was occurring from particulate organic matter. Groundwater at the same site had on average more than three times as much DOC as the surface

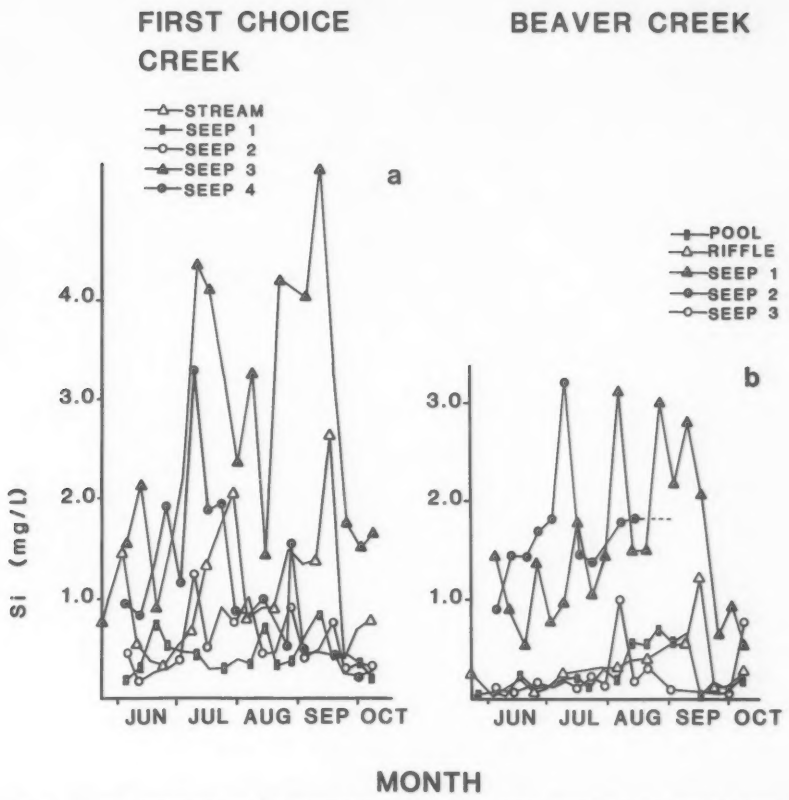

FIG. 4. Silicon concentrations for groundwater and surface water samples taken weekly from First Choice Creek and Beaver Creek catchments from May to October 1984.

water. If this groundwater pool is volumetrically large and flow is sufficiently high, then it could be a major source for surface water DOC.

Considerable differences in DOC concentration between groundwater and surface water suggest that large amounts of organic carbon may be removed either at or below the sediment–water interface. Hynes (1983) has suggested that the inflow of organic matter from groundwater may be both reduced and modified by a biologically active zone (the hyporheic zone) below the sediment–water interface. This is supported by Bretschko (1981) and Leichtfried (1985, 1988) who found a maximum abundance of detritivorous fauna at 30 cm below the sediment surface in a mountain stream. These animals prefer particulate organic matter colonized by fungi and bacteria; hence, a rich biota is associated with these sediments.

Wallis et al. (1981) provided evidence suggesting that about half the DOC in groundwater is lost upon entering a stream. They hypothesized that the loss was due to exposure of groundwater DOC to benthic bacteria as it seeped through the sediments. Carbon may be actively removed by microbial communities in the top sediment layers and at the sediment–water interface (Ladd et al. 1982) and, in some cases, there is considerable evidence for high levels of microbial activity at the benthic boundary layer (Lock and Hynes 1975; Dahm 1981; Mickleburgh et al. 1984).

If we assume that groundwater DOC sampled at the farthest site downstream is representative of the groundwater table, then potentially 70% of the DOC in groundwater was removed within the hyporheic zone or at the sediment–water interface. This assumption is based on three factors: (1) in the absence of precipitation, groundwater was the only source of stream flow in First Choice Creek; (2) groundwater at the Beaver Creek lysimeter sites has considerably higher concentrations of DOC than those measured at the upper sites in First Choice Creek; and (3) groundwater at the upper sites in First Choice Creek was sampled from lysimeters placed in actively flowing springs. Groundwater at these sites was well oxygenated and considerable microbial uptake/degradation of DOC may have occurred before sampling. This suggests that biotic or abiotic activity in the sediments could sequester a major proportion of allochthonous organic carbon available to the stream system. Measurement of epilithic microbial production in this stream was low (9.2 g $C \cdot m^{-2} \cdot yr^{-1}$, Naiman et al. 1987), suggesting that microbially labile components of the groundwater DOC were removed within the sediments and never reached the surface water.

Extensive carbon budgets have been constructed for both First Choice Creek and Beaver Creek (Naiman et al. 1987). However, contributions from groundwater sources were not considered. In order to evaluate groundwater inputs, knowledge of flow rates is necessary. As groundwater hydrology for this area is not known, an estimation of groundwater carbon input to Beaver Creek is impossible. However, a rough estimate of the potential input can be made for First Choice Creek, since

groundwater is the only source of stream flow. This estimate is based on a number of assumptions but serves to show the possible magnitude of organic carbon inputs from groundwater. Assuming a mean concentration of 2.45 mg C/L in groundwater (mean of seeps 1–4, skewed by seep 4, see above for justification) and 1.38 mg C/L in surface water, then 1.07 mg C/L is removed by the sediments. The mean annual discharge for First Choice Creek is 4.1×10^5 m³/yr, resulting in an input of 4.4×10^5 g C/yr for the total stream or about 3525 g $C \cdot m^{-2} \cdot yr^{-1}$. The measured input of organic carbon from all other sources is 416.7 g $C \cdot m^{-2} \cdot yr^{-1}$ (Naiman et al. 1987), which is only 11% of the total if that retained from the groundwater is included. Extensive hydrological analysis of groundwater flow, together with measurements of mineralization rates in the deeper sediments, will be necessary to determine the contribution of groundwater organic carbon to surface water budgets and identify more precisely the lower boundary to the stream ecosystem.

DOC concentrations in Beaver Creek show no relationship down the length of the stream, with no apparent difference between groundwater and surface water at seeps 1 and 3, suggesting rapid exchange between groundwater and surface water in this stream. Nevertheless, for most of the summer, seep 2 groundwater had greatly elevated DOC concentration over surface water, suggesting a slow rate of exchange. This is supported by the fact that only very small samples of groundwater could be collected. Closer examination of the soil type, depth, drainage area, and groundwater flow would be necessary to indicate why this site differed from other sites.

In contrast with surface water where nitrate is the dominant form of nitrogen, groundwater nitrogen was generally in the form of ammonium, indicating reduced, probably anoxic, conditions (Wetzel 1983). In First Choice Creek, significant differences between surface water and groundwater began to occur at downstream seeps 3 and 4 with more than 10-fold higher ammonium concentrations in groundwater over surface water. Surface water and groundwater at upstream seeps 1 and 2 had considerably higher nitrate to ammonium ratios than groundwater at seeps 3 and 4 ($\bar{x} = 2.2$ and $\bar{x} = 0.1$, respectively; $t = 3.9$, $p < 0.001$), presumably due to the oxidized nature of the spring water and surface water. This suggests that there is variation in the depth of the aerobic/anaerobic boundary within the sediments. Unfortunately, redox potentials were not measured in sediment profiles. A flux of ammonium nitrogen across this boundary provides ideal conditions for nitrifying bacteria (Wetzel 1983). As a result of this flux of available nitrogen, a large microbial population can be supported in the surface layers of the sediments, despite low levels of available forms of nitrogen in the surface waters.

Further evidence for this is provided by the Beaver Creek nutrient data. Total nitrogen concentrations were significantly lower in surface water than in groundwater, and nitrate to ammonium ratios were significantly higher for surface water than for groundwater ($\bar{x} = 2.6$ and $\bar{x} = 0.03$, respectively; $t = 8.9$, $p < 0.001$). A separate study (Ford and Naiman 1988) has shown considerable methane evasion from accumulated sediments of beaver ponds associated with this system. Production of methane in sediments often results in an active community of methane-oxidizing bacteria at the sediment–water interface (Lidstrom and Somers 1984), and these bacteria obligately oxidize ammonium to nitrate (O'Neill and Wilkinson 1977). Therefore, we suggest that extensive microbial activity at the sediment–water interface may be responsible for the large discrepancy between nitrogen levels and nitrate to ammonium ratios between surface and groundwaters. Ammonium is an energy-efficient source of nitrogen for plants (Wetzel 1983); thus, assimilation of ammonium by cyanobacteria and diatoms at the benthic boundary layer may be important (Jansson 1980).

Low levels of phosphorus are to be expected in groundwaters due to the low solubility of phosphate-containing minerals (Wetzel 1983). We observed low phosphorus levels, with a significant reduction between groundwaters and surface water in Beaver Creek. High levels of microbial activity at the sediment–water interface would utilize phosphorus with a corresponding reduction of concentrations between groundwater and surface water, particularly if phosphorus is a limiting nutrient in these systems, as suggested by Chow-Fraser and Duthie (1983). However, in First Choice Creek there was a significant increase in phosphorus concentration between groundwater and surface water with a concomitant increase in the ratio of orthophosphate to soluble total phosphorus. This suggests that phosphorus is not a limiting nutrient in First Choice Creek. Higher soluble total phosphorus in the surface water compared with groundwater could be explained by concentration in the sediments and regeneration to the water column. High levels of phosphorus may be adsorbed to the surface layers of the sediment by sedimentation, as has been seen in lakes (e.g. Sommers et al. 1970; Otsuki and Wetzel 1972). Phosphorus may be retained in the sediments by an oxidized microzone at the sediment–water interface (Mortimer 1971) and regenerated to the water column by activity of detritivores at the benthic boundary layer.

Groundwater has been shown to be an important source of silicon for diatom production in lake systems (Hurley et al. 1985). The average groundwater silicon concentration for Beaver Creek is three times the surface water concentration and hence may be important in diatom production. Relatively high levels of silicon in First Choice Creek surface water may be the result of sloughing off of diatomaceous slimes from rock surfaces which are then microbially degraded. First Choice Creek has virtually no sediment load (Naiman 1982), and hence, diatom communities readily develop on hard substrates and are responsible for 39% of the gross primary production in this stream (Naiman 1983a).

In conclusion, considerable variation exists in nutrient loading between different groundwater sites. Exact budgetary analysis requires extensive knowledge of sediment metabolism rates, groundwater hydrology, and nutrient dynamics occurring across active boundaries (Naiman et al. 1988). However, the data presented here suggest that groundwater is potentially an important source of DOC, nitrogen, and silicon for boreal forest streams. Ammonium and nitrate data indicate a redox change, suggesting oxidative, biotic processes near the sediment – surface water interface. It is apparent that the boundary layer occurring between groundwater and surface water processes substantial amounts of organic carbon and inorganic nutrients and needs to be more carefully considered in budgetary analysis of stream ecosystems.

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Trace Element Analysis of Fish Sagittae as an Aid to Stock Identification: Pink Snapper (*Chrysophrys auratus*) in Western Australian Waters

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Edmonds, J. S., M. J. Moran, N. Caputi, and M. Morita. 1989. Trace element analysis of fish sagittae as an aid to stock identification: pink snapper (*Chrysophrys auratus*) in Western Australian waters. Can. J. Fish. Aquat. Sci. 46: 50–54.

Concentrations of trace elements in the sagittal otoliths of pink snapper (*Chrysophrys auratus*) from seven locations along the coast of Western Australia were measured by inductively coupled plasma atomic emission spectrometry (ICP-AES). Canonical variate (discriminant) analyses of the concentrations of eight elements (Na, Mg, Si, P, S, K, Fe, and Sr) from all fish when corrected for fish length showed the pattern of concentrations to be specific to the location of capture of the fish. Trace elements are likely to be irreversibly deposited in the sagittae during growth and should provide an integrated environmental history of each fish. Differences in elemental composition may thus be useful for identifying nonmixing groups of fish that may be regarded as separate stocks for some fisheries management purposes.

On a mesuré les concentrations des éléments à l'état de trace dans les sagittas (l'un des otolithes) de la dorade royale (*Chrysophrys auratus*) en sept emplacements le long de la côte de l'ouest de l'Australie. Les mesures ont été effectuées par spectroscopie d'émission avec plasma induit par haute fréquence (SE/PIHF). L'analyse (discriminante) des variables canoniques des concentrations de huit éléments (Na, Mg, Si, P, S, K, Fe, Sr) mesurées dans tous les poissons capturés a montré que ces concentrations étaient propres aux lieux de capture des poissons. Les éléments à l'état de trace sont susceptibles de se déposer en permanence dans les sagittas des poissons au cours de leur croissance et devraient permettre de faire l'historique écologique de chacun d'entre eux. À certaines fins, en matière de gestion des stocks, les différences dans la composition élémentaire pourraient donc faciliter l'identification des groupes de poisson ne se mêlant pas qui peuvent être considérés comme des stocks distincts.

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One of the major requirements of effective fisheries management is an ability to distinguish the separate stocks that make up the total population of a single fish species (Rounsefell 1975). The populations of some species form discrete units which can be regarded as separate breeding stocks; these can sometimes be identified by the determination of genetic differences revealed by the electrophoretic examination of soluble proteins (Shaklee 1983). Other populations cannot be so conveniently divided into discrete units as they are continuously distributed, sometimes over a very large region. When the range of movement of individual fish is small in relation to the total range occupied by such a population, management may still be more realistically directed at smaller subsections of the population rather than the total distribution. This is especially so if attributes such as growth and mortality rates vary greatly from one area to another, or if events such as drastic overfishing in one area have negligible effects on the stocks in other parts of the population range. Techniques such as tagging (Lear 1984), morphometric studies (Dempson and Misra 1984), and use of parasites as biological markers (MacKenzie 1983) have been employed to distinguish such nonmixing localised stocks.

In Western Australia, pink snapper (*Chrysophrys auratus*) are distributed from the south coast (latitude 35°S) along 2000 km of coastline to a northern limit at about 21°S (Fig. 1). Although the species is commercially exploited to varying extents along its entire range, the major fishing area is in Shark Bay (Fig. 1, inset). Increasing fishing pressure in this locality has resulted in management problems which necessitated a knowledge of the number and discreteness of the stocks which make up the population. Tagging (M. J. Moran et al., unpubl. data) and electrophoretic studies (Johnson et al. 1986) have both indicated that snapper in the Shark Bay area may exist in at least three separate, essentially nonmixing stocks.

In the study reported here, we have sought to identify separate stocks within the total population of pink snapper by trace element analyses of the sagittae (the largest of three, paired, calcium carbonate otoliths in the auditory/balance system) of individual fish. Trace elements contained within the sagittae were assumed to have been irreversibly deposited during the life of the fish and would consequently provide an integrated

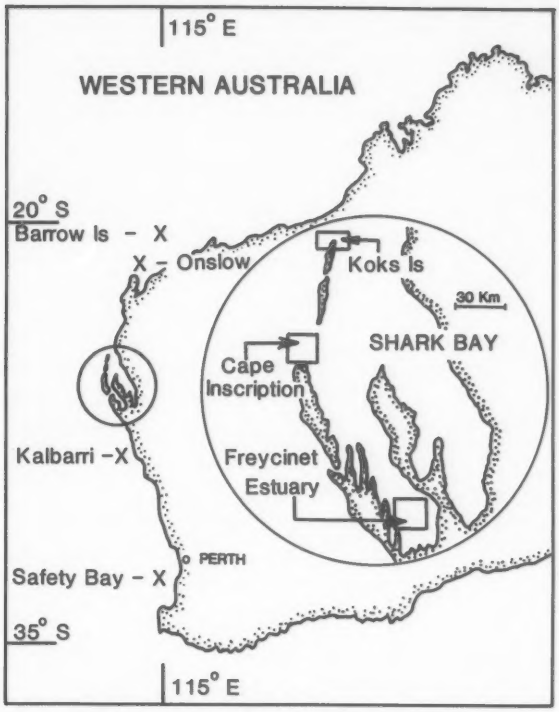

FIG. 1. Map of Western Australia with enlargement of Shark Bay area (inset) showing the seven locations sampled in this study.

environmental history of each fish. Elements included in sagittae are not reworked after deposition, unlike the constituents of cellular bone (Mulligan et al. 1987). This stability is the basis for several techniques used to validate age determination in fish, for example, measurement of $^{210}Pb/^{226}Ra$ ratios in sagittae (Bennett et al. 1982) and use of tetracycline which binds to calcium at the time of deposition and forms a fixed time mark on the sagitta (Beamish and McFarlane 1987). Clear discrimination of groups of fish on such a basis would then suggest that they might be regarded as separate, nonmixing stocks for some fisheries management purposes, for example, the application of yield-per-recruit models (Beverton and Holt 1957) or determination of appropriate spatial scales for sampling of population parameters or reservation of unfished sanctuary areas for conservation.

There have been only a few previous attempts at stock identification by trace element analysis of hard tissues (Calaprice 1971; Lapi and Mulligan 1981; Mulligan et al. 1983). The only study to have employed sagittae was that of Mulligan et al. (1987), in which energy-dispersive X-ray analysis was used to determine presence or absence of various elements.

Materials and Methods

Material was analysed from one (randomly chosen) of the pair of sagittae located in the otic cavity of the snapper, posterior and dorsal to the brain. The sagittae were easily removed and cleaned and were transported to the laboratory without freezing or special equipment. Each sagitta (approximate weight 300 mg) was thoroughly scrubbed with a nylon brush in distilled water, rinsed with distilled water, air dried, ground to a fine powder, dried in vacuo at 110°C for 2 h, and a portion (125 mg) dissolved in hydrochloric acid (5 mL, 12 N), diluted to 50 mL, and analysed by inductively coupled plasma atomic emission spectrometry (ICP-AES) using Daini – Seiko JY48PV or JY38P instrumentation. Matrix matching was employed to correct for the matrix effect of the high concentrations of Ca in the samples. Nine elements (Na, Mg, Si, P, S, K. Ca, Fe, and Sr) were present in sufficient concentrations to provide useful data. All of these, with the exception of Ca (38.33 ± 0.33% of all samples), were used in the statistical analysis.

Results

Analysis of variance of the concentration of eight elements with location as a factor and length as a covariate resulted in seven of the elements showing a significant inverse relationship with length (Fe, K, Mg, Na, and Si at the 0.001 level and P and S at the 0.05 level). The concentration of Sr did not show any significant variation with length. Location was significant for all elements at the 0.001 level, except for Mg (0.01 level) and Fe (0.05 level). However, there was still considerable overlap in the ranges of the elemental compositions between locations when the elements were examined individually (Table 1).

Canonical variate (discriminant) analyses (CV analyses) (Seal 1964) using the eight elements listed above were performed to determine the differences in composition of the sagittae of snapper from the seven locations, including three from Shark Bay (Fig. 1). Concentrations of the seven elements showing a significant inverse relationship with length of fish were each corrected for size as follows:

$$AC = C - rL$$

where C is the concentration of a given element for a fish of length L, r is the regression coefficient for the covariate length in the above analysis of covariance, and AC is the concentration adjusted for length.

An initial CV analysis contained the samples from all seven locations. To examine the differences within Shark Bay in more detail, a separate CV analysis was carried out on only these three locations. As a further example, a separate CV analysis was also carried out on the three northernmost locations: Koks Island, Onslow, and Barrow Island.

The CV analysis for the seven locations showed a separation of the Freycinet sample from the other locations, while the Koks Island, Cape Inscription, and Kalbarri samples were separated out on the second function (Fig. 2a). The third CV function separated the Safety Bay samples from all other locations (Fig. 2b). Table 2a shows the standardised CV functions for this analysis.

The separate CV analysis for the three Shark Bay locations showed that there was also a separation of fish from Koks Island and Cape Inscription (Fig. 3a). Sagittae from Freycinet fish had higher levels of Si and Sr compared with those from other fish, while those from Koks Island had high S concentrations (Table 2).

An unbiased estimate of the correct classification rate for the fish in the three Shark Bay locations was obtained by repeating the CV analysis using only 90% of specimens and then using these CV functions to classify the remaining 10% of specimens. This analysis was repeated 10 times resulting in 84% of specimens being correctly classified.

TABLE 1. Length (snout to caudal fork, mm) and trace element composition (ppm) of pink snapper sagittae from seven locations along the Western Australian coast. Detection Limits (ppm): Mg, Sr = 1; Fe = 5; Si = 10; Na, P, S = 100; K = 200. If an element was undetected in a particular sample, its concentration was taken as 0 for the purpose of calculating the mean concentraton for the location. This has occasionally resulted in means of less than the detection limit. If an element was undetected in all samples from a particular location, the mean concentration for that element is recorded as 0.

			Shark Bay				
	Barrow Island	Onslow	Koks Island	Cape Inscription	Freycinet	Kalbarri	Safety Bay
Sample size	14	20	16	23	52	31	21
Length							
Mean	567	514	354	424	391	572	475
SD	41	39	46	62	112	124	122
Range	514 – 654	432 – 581	274 – 441	326 – 567	207 – 610	317 – 777	362 – 855
Na Mean	2188	2290	2455	2340	2404	2141	2462
SD	94	139	115	119	174	149	138
Range	2034 – 2370	1874 – 2548	2281 – 2763	2146 – 2612	2077 – 2783	1854 – 2509	2250 – 2787
Mg Mean	8	11	11	9	10	8	10
SD	1	3	2	3	3	3	4
Range	5 – 10	7 – 20	8 – 15	4 – 16	5 – 21	4 – 18	6 – 20
Si Mean	18	34	6	0	12	2	14
SD	16	17	7	0	9	11	13
Range	0 – 43	0 – 69	0 – 21	0	0 – 27	0 – 60	0 – 36
P Mean	111	131	137	124	159	110	80
SD	22	25	20	19	30	24	55
Range	77 – 172	83 – 169	97 – 165	97 – 169	105 – 212	78 – 190	12 – 192
S Mean	174	183	345	238	310	226	161
SD	49	65	44	64	67	53	97
Range	90 – 257	69 – 270	257 – 404	25 – 329	184 – 449	79 – 363	13 – 352
K Mean	389	375	436	410	468	350	416
SD	53	28	34	41	80	38	47
Range	278 – 464	327 – 430	366 – 518	310 – 486	316 – 602	272 – 424	336 – 500
Fe Mean	0	0.3	3	2	4	0	1
SD	0	1.1	5	5	6	0	2
Range	0	0 – 5	0 – 13	0 – 21	0 – 26	0	0 – 7
Sr Mean	2159	2093	2102	1996	2615	2054	2115
SD	144	164	143	170	315	206	198
Range	1886 – 2400	1866 – 2488	1801 – 2294	1598 – 2236	2056 – 3957	1682 – 2524	1801 – 2538

TABLE 2. Standardised canonical variate functions for the concentrations of eight elements in the sagittae of pink snapper from (a) all seven locations (Fig. 2) and (b) the three locations in Shark Bay (Fig. 3a).

		Canonical variate		
Element		I	II	III
(a)	Na	0.25	0.28	−0.93
	Mg	−0.16	0.04	0.52
	Si	−0.58	0.74	0.11
	P	0.19	0.38	0.44
	S	0.25	−0.59	0.42
	K	0.35	−0.31	−0.32
	Fe	0.16	0.05	−0.04
	Sr	0.55	0.64	0.00
(b)	Na	−0.15	−0.18	
	Mg	0.16	0.66	
	Si	0.45	0.13	
	P	0.23	−0.03	
	S	−0.32	1.20	
	K	−0.03	−0.53	
	Fe	0.07	−0.34	
	Sr	0.85	−0.06	

The CV analysis of the three northern locations (Koks Island, Onslow, and Barrow Island) showed a significant separation of fish (Fig. 3b). The latter two locations were only 50 km apart.

Discussion

Electrophoretic studies have indicated that the Freycinet snapper are genetically distinct from the other Shark Bay stocks (Johnson et al. 1986). Tagging has revealed no mixing of fish between Freycinet and any other stock, while there is limited mixing between Koks Island, Cape Inscription, and Kalbarri (M. J. Moran et al., unpubl. data). The separation indicated by the trace element composition is therefore compatible with the results of the tagging study.

The reasons for differences in the trace element levels of sagittae of fish from different locations are currently unknown but may reflect ambient water or sediment levels, dietary differences, or water temperature differences (Gauldie et al. 1980). Differences in trace elements would seem unlikely to be a result of genetic variability (Behrens-Yamada et al. 1987) and will not, without additional evidence, identify separate breeding stocks. No single technique currently used for stock discrimination tells the fishery manager all he needs to know. For exam-

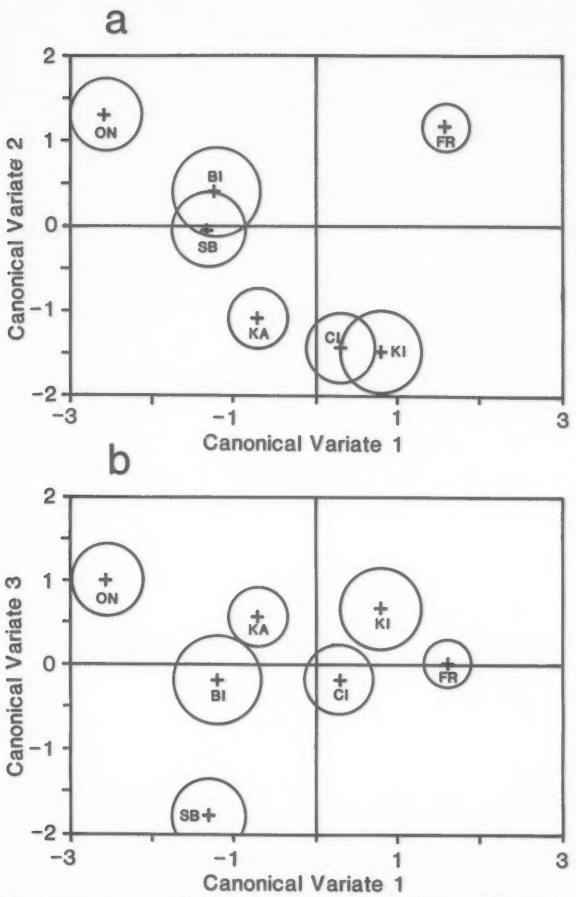

FIG. 2. Mean values of the first three canonical variates (CV) based on eight element concentrations in the sagittae of pink snapper from Cape Inscription (CI), Freycinet Estuary (FR), Koks Island (KI), Safety Bay (SB), Kalbarri (KA), Onslow (ON), and Barrow Island (BI). The 95% confidence circles about the means (Seal 1964) are shown. (a) CV1 versus CV2; (b) CV1 versus CV3.

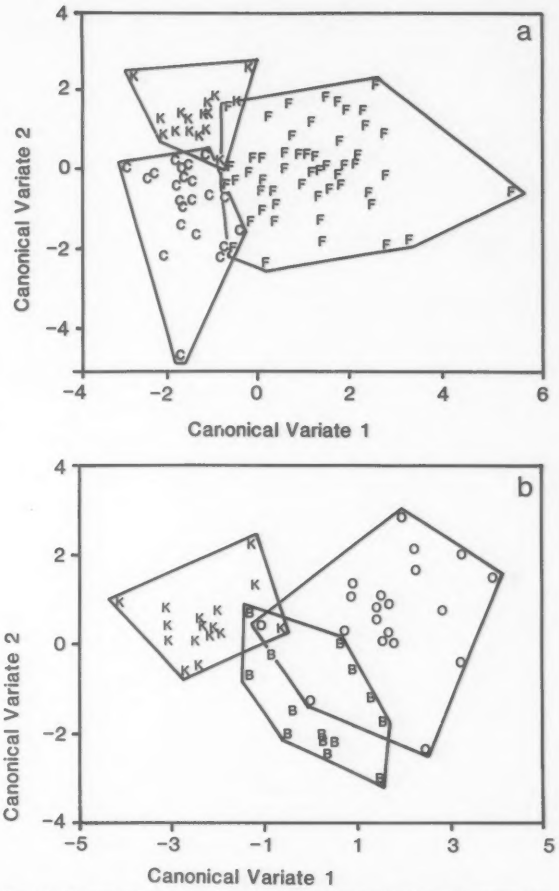

FIG. 3. Canonical variates 1 and 2 based on eight element concentrations in the sagittae of pink snapper (a) from three locations in Shark Bay: Cape Inscription (C), Freycinet Estuary (F), and Koks Island (K); and (b) from the three northernmost locations: Koks Island (K), Barrow Island (B), and Onslow (O). A boundary has been drawn around each group of data points to show their approximate extent.

ple, studies of genetic differences may not be sufficiently sensitive for fishery management purposes, i.e. the small amount of mixing between populations that is needed to maintain genetic homogeneity is negligible by fishery management standards. Another set of techniques including tagging, biological markers such as parasites, and morphometrics all give indications of the degree of mixing between groups of postlarval fish but give no information regarding the exchange of larvae between the groups. The trace element composition of sagittae will supply similar information to this second set of methods. The chemical method does have certain advantages, for example it can produce results much more quickly than a tagging study; also, it does not depend upon the distribution of fishing effort. Examination of the sagittae to a greater degree of resolution, i.e. by independent trace element analyses of the core and the various concentric layers of the intact sagitta, may reveal the environmental history of the fish in considerable detail. Currently we are working on this aspect of the study and to extend the technique to a range of other fish species.

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Oxygen Availability Provides a Signal for Hatching in the Rainbow Trout (*Salmo gairdneri*) Embryo

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Latham, K. E., and J. J. Just. 1989. Oxygen availability provides a signal for hatching in the rainbow trout (*Salmo gairdneri*) embryo. Can. J. Fish. Aquat. Sci. 46: 55–58.

The hatching of rainbow trout (*Salmo gairdneri*) embryos can be stimulated by subjecting them to low oxygen partial pressures (P_{O_2}) during the final days of incubation or delayed by elevating P_{O_2}. Most embryos develop the ability to hatch between days 26 and 27 of incubation at 12°C. During this time, similar hatching frequencies are obtained at any P_{O_2} below a critical value in the 94–135 mm Hg (6.5–9.3 mg/L) range (1 mm Hg = 133.322 Pa). The P_{O_2} required for continued incubation increases with developmental time such that hatching cannot be delayed beyond day 30 at 12°C. These results indicate that oxygen availability influences the time at which trout embryos hatch and that hatching occurs when hatchable embryos are confronted with an ambient P_{O_2} that is inadequate to satisfy aerobic metabolic requirements.

L'éclosion des embryons de truites arc-en-ciel (*Salmo gairdneri*) peut être stimulée en soumettant ceux-ci à de faibles pressions partielles d'oxygène pendant les derniers jours d'incubation ou retardée en élevant la teneur en P_{O_2}. La plupart des embryons sont capables d'éclore entre les 26e et 27e jours d'incubation à 12°C. Pendant ce temps, des fréquences d'éclosion semblables sont obtenues à n'importe quelle teneur de P_{O_2} en-deçà d'une valeur critique dans la gamme de 94–135 mm Hg (6.5–9.3 mg/L) (1 mm Hg = 133.322 Pa). Comme le P_{O_2} nécessaire au prolongement de l'incubation augmente avec le temps de développement, l'incubation ne peut être retardée au-delà du 30e jour à 12°C. Ces résultats révèlent que la disponibilité de l'oxygène influe sur le temps qu'il faut aux embryons de truites pour éclore et que l'éclosion se produit lorsque les embryons éclosables sont mis en présence d'une teneur ambiante de P_{O_2} insuffisante pour satisfaire les besoins métaboliques aérobies.

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Although the physical and chemical mechanisms of teleostean hatching have been studied, the stimulus for hatching has not been clearly defined (see review by Yamagami 1981). Iuchi et al. (1985) describe two categories of artificial hatching stimuli. The first category includes stimuli and agents related to embryonic respiration and metabolism such as hypoxia and cyanide poisons. The second includes other stimuli such as electric current, hormones, and ionophores which are thought to act directly upon the hatching glands. The first group of stimulants clearly points to a relationship between hatching and embryonic respiration, suggesting that O_2 availability could be the normal hatching stimulus. Several fish, such as the so-called "annual fishes" (Myers 1952; Harrington 1959; Peters 1963; Pollard 1971; Wourms 1972) and the killifish (*Fundulus heteroclitus*) (DiMichele and Taylor 1980), breed in an unusual manner by depositing eggs in locations where, following fertilization, they endure a period either exposed to air or buried in soil. Hatching in these species occurs upon immersion in water, from which O_2 might be less easily obtained. Low O_2 concentrations interrupt diapause in some annual fishes (Peters 1963) and are involved in stimulating embryos of *F. heteroclitus* to hatch (DiMichelle and Taylor 1980). For teleosts with more typical breeding habits, delayed hatching is correlated with high O_2 concentrations (Bay 1966; Gulidov 1971; Hagenmaier 1972; vonBaumgarten et al. 1975) and precocious hatching with low O_2 concentrations (Alderdice et al. 1958; Garside 1959). In the latter instances, however, the O_2 concentrations that brought about hatching were either not indicated or were applied for periods long enough to affect the rate of development of the embryos. The present experiment seeks to define the relationship between O_2 availability and teleost hatching more precisely by determining oxygen partial pressure (P_{O_2}) at which hatching occurs and the time during incubation at which hatching can first be elicited by hypoxia.

Materials and Methods

Adult rainbow trout (*Salmo gairdneri*) were obtained from Wytheville, Virginia, and artificially spawned as described by Lietritz and Lewis (1976). The eggs were incubated in a single layer at 12–13°C in 500 mL of aerated artificial spring water (Birge et al. 1978). The water was changed and dead eggs removed at 4-d intervals. Under these conditions, hatching typically occurs on day 27 of incubation.

All experiments were performed at 12°C in artificial spring water. Experimental and control groups were transferred to separate Erlenmeyer flasks containing 150 mL of water equilibrated with the appropriate mixture of O_2 and N_2 at 12°C and incubated in a 12°C water bath. Each group consisted of fewer than 20 eggs to minimize the possible effects of released hatch-

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TABLE 1. Effects of diminished O_2 concentrations on hatching of rainbow trout embryos. All experiments listed were performed on day 26 of incubation at 12–13°C. The percent hatching was determined after 2 h of exposure to each P_{O_2}.

P_{O_2} (mm Hg)	Number of embryos	% hatching
135	35	0
151	45	0
94	16	50*
151	20	0
47	37	56*
151	39	1.8
6	30	56*
151	29	0

*Significantly different from controls (151 mm Hg) in a percentage test ($p < 0.0001$).

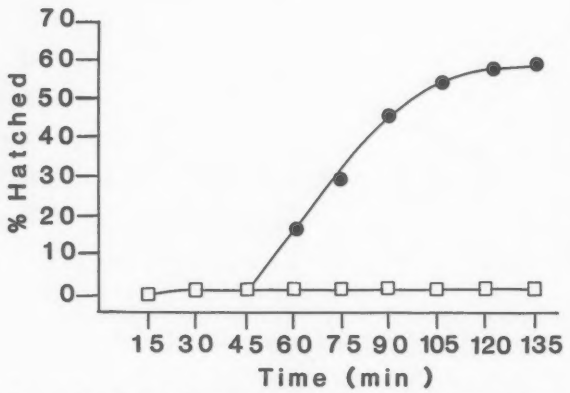

FIG. 1. Cumulative hatching of rainbow trout embryos during 135 min of exposure to low P_{O_2}. ●, 98 embryos exposed to a P_{O_2} of 94 mm Hg or less; □, 133 control embryos remaining at a P_{O_2} of 151 mm Hg. All experiments were performed on the 26th day of incubation of 12–13°C.

ing enzyme upon unhatched eggs. Flasks were sealed with stoppers through which 5-mm-diameter glass tubing was passed for a constant gas supply. Gas mixtures were made using Matheson flow meters and the P_{O_2} at equilibrium were measured with an oxygen electrode (Radiometer). Hatching was monitored by recording the number of hatched embryos in each flask at 15-min intervals for up to 135 min. Results are expressed as the percent hatching observed at each P_{O_2} and levels of significance analysed using a percentage test (Sokal and Rohlf 1969).

For those experiments designed to determine whether hatching could be delayed by elevating the P_{O_2}, 141 eggs were transferred on day 25 to water equilibrated at P_{O_2} ranging from 257 to 690 mm Hg (1 mm Hg = 133.322 Pa) for further incubation. The number of hatched embryos was recorded at 24-h intervals for each P_{O_2}. The significance of delay was evaluated using a chi-square test (Everitt 1977).

Results

Experiments began 16 d after fertilization. Of the 100 embryos tested between days 16 and 24, no hatching was observed at a P_{O_2} of 47 mm Hg (3.2 mg/L). On day 25, 9 of

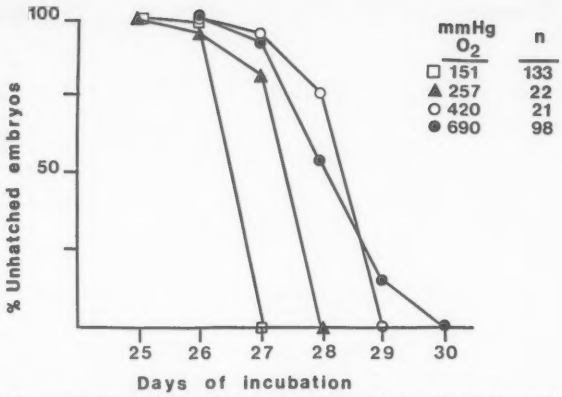

FIG. 2. Effects of elevated P_{O_2} on the time of hatching of rainbow trout embryos. Embryos were transferred to the elevated P_{O_2} indicated on day 25 of incubation (12–13°C). The percentage of hatched embryos was recorded at 24-h intervals thereafter. Hatching was delayed at elevated P_{O_2}, with the length of delay proportional to the P_{O_2} provided. Hatching cannot be delayed beyond day 30 at 12°C, even with a near-saturating P_{O_2} of 690 mm Hg.

47 embryos hatched during a 3-h exposure to this P_{O_2} as compared with less than 0.1% hatching in the stocks remaining at atmospheric P_{O_2} (151 mm Hg, 11 mg/L). On day 26, hatching could be induced readily and nearly 100 embryos were tested in a range of P_{O_2} (Table 1). Of the 133 embryos serving as controls at a P_{O_2} of 151 mm Hg, only 1 hatched. By contrast, hatching frequencies of 50–56% were obtained for groups exposed to P_{O_2} of 6, 47, and 94 mm Hg (0.44, 3.2, and 6.5 mg/L, respectively) for 2 h. No hatching occurred at a P_{O_2} of 135 mm Hg (9.3 mg/L), indicating that the critical P_{O_2} for hatching on day 26 lies between 94 and 135 mm Hg.

The similarity of hatching frequencies on day 26 at all P_{O_2} of 94 mm Hg or less indicated that any P_{O_2} below some critical P_{O_2} in the range of 94–135 mm Hg was sufficient to elicit hatching in all of the hatchable embryos present. To determine whether the observed hatching frequencies (Table 1) indeed reflected the maximum response to reduced P_{O_2}, the values for cumulative percent hatching, recorded at 15-min intervals during each experiment, were examined (Fig. 1). Since, the total percent hatching obtained was very similar at P_{O_2} of 6, 47, and 94 mm Hg, the data for these experiments were combined. No hatching occurred during the first 45 min of exposure to reduced P_{O_2}. Subsequently, the percentage of hatched embryos increased rapidly, reaching 45% by 90 min and 56% by 2 h of treatment. Further exposure to reduced P_{O_2} for longer than 2 h increased the percentage of hatched embryos (59%) only slightly. These data clearly demonstrate that virtually all of the hatchable embryos present respond during 2 h of treatment and support the conclusion that any P_{O_2} below the critical P_{O_2} induces hatching in all of the hatchable embryos. Since 100% hatching could be induced by these same low P_{O_2} values on day 27, the 41% of the embryos which did not hatch on day 26 were probably embryos which had not yet developed the ability to hatch rather than embryos which had succumbed to some lethal effect of hypoxia.

Hatching was significantly ($p < 0.001$) delayed in embryos incubated at elevated P_{O_2} (Fig. 2). The length of delay was directly related to the P_{O_2} provided, although there was no significant difference between 690 mm Hg (47.3 mg/L) and 420 mm Hg (28.7 mg/L). At both 420 and 690 mm Hg, hatching

was delayed for 2 d beyond the time of hatching observed for embryos remaining at atmospheric Po_2 (151 mm Hg). Hatching in a few embryos (16%) was delayed for 3 d using a Po_2 of 690 mm Hg. Embryos that had been given elevated Po_2 on day 25 hatched when subjected to a Po_2 of 6 mm Hg on days 26–30 and hatched at 151 mm Hg on day 27 and thereafter. This is consistent with hatching of the untreated embryos, indicating that no reduction in hatchability occurred during incubation at elevated Po_2.

Discussion

Rainbow trout embryos hatch prematurely when subjected to decreased Po_2 shortly before their expected hatching time. Premature hatching can be induced in this manner only near the end of incubation, indicating concurrent development of hatchability, increased O_2 consumption, or both. The data obtained on day 26 of incubation illustrate rather uniform hatching frequencies at all Po_2 of 94 mm Hg or less. Previously, Alderdice et al. (1958) indicated that the amount of hatching of chum salmon (*Oncorhynchus keta*) embryos varied with the degree to which Po_2 was reduced. These researchers, however, subjected eggs to low O_2 concentrations continuously for 7 d (about 12% of the average incubation time) and found that the metabolic rate of embryos varied with O_2 availability. This variation in metabolic rate, which apparently involved a decrease in the rate of development, would lead to variation in the capacity to tolerate a low Po_2 and in the ability to hatch. Our results were obtained with a brief 2-h exposure during the final days of incubation. During this 2-h period, which represents less than 0.03% of the total incubation time, any hatching that is observed must represent the immediate response of all hatchable embryos to the reduced Po_2. The similarity of the hatching frequencies observed suggests that a critical minimum Po_2 in the 94–135 mm Hg range must be required for continued incubation.

The absence of a sufficiently high Po_2 is expected to constitute a potent stimulus for hatching. Since the zona radiata retards O_2 passage (Hayes et al. 1951), the minimum ambient Po_2 necessary to provide for embryonal O_2 consumption must be higher for the unhatched animal. If the ambient Po_2 falls below this minimum, hatching would allow the embryo to escape the hypoxic conditions that would arise within the egg as a consequence of insufficient diffusion of O_2 across the zona radiata. Alternatively, any Po_2 above this minimum would be compatible with continued incubation, and a Po_2 above atmospheric would cause delayed hatching. The results shown in Fig. 2 indicate no reduction in hatchability and confirm this permissive role of a high Po_2. Since O_2 consumption increases with development (Scott 1916; Hayes et al. 1951), it is expected that the critical Po_2 for stimulating hatching also increases with development. This is supported by the fact that hatching occurred at subatmospheric Po_2 on days 25 and 26, at 151 mm Hg on day 27, and at progressively higher Po_2 from day 28 to day 30.

It should also be noted that both photoperiod and temperature have recently been reported to affect the time of hatching in Atlantic salmon (*Salmo salar*) embryos (Brannas 1987). Hatching during daylight hours in embryos given a photoperiod with extended daylight (16 h light : 8 h dark) was assumed to be related to a negative phototactic behavioral response. A behavioral response such as this might well affect the metabolic rate of the embryos. Physical agitation also affects teleost hatching (Næsje and Jonson 1988). Given the effect of O_2 availability upon hatching reported here, it is likely that photoperiod or any other factor which affects metabolic rate, such as temperature or perhaps physical agitation, would also influence hatching by causing alterations in the rate of embryonic O_2 consumption and hence the Po_2 required for continued incubation.

The observed response to hypoxia is not unique to fish eggs. Similar responses have been reported for amphibian eggs (Petranka et al. 1982) and for eggs of certain aquatically breeding invertebrates such as mosquitos (Gjullin et al. 1941; Gillet et al. 1977; Kardatzke 1979). Future investigation may reveal that hypoxia is a common stimulus for hatching, particularly in aquatic habitats where diminished O_2 concentrations occur and atmospheric Po_2 may become insufficient to adequately supply O_2 for metabolic demands.

Acknowledgments

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Phosphorus Reservoirs in the St. Lawrence Upper Estuary

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Lucotte, M. 1989. Phosphorus reservoirs in the St. Lawrence Upper Estuary. Can. J. Fish. Aquat. Sci. 46: 59–65.

A first-order phosphorus (P) budget is presented for the St. Lawrence Upper Estuary. The upwelled intermediate waters of the Laurentian Trough contribute 90% of the dissolved P source to the estuary whereas the St. Lawrence River carries in most of the particulate P. The dissolved P flux to the estuary (~80 000 t·yr^{-1}) is about 10 times larger than the particulate one (~5000 t·yr^{-1}, more than half being detrital apatite). However, the dissolved and particulate estuarine P reservoirs are of the same order of magnitude (~3000 t) because of the short residence time of the water masses relative to that of the solid matter. This rapid water mass turnover is responsible for the conservative behavior of dissolved orthophosphate (PO_4) throughout the estuary; dissolved PO_4 concentrations are not significantly affected by the biogeochemical exchange reactions involving particulate P. Approximately 500 and 300 t PO_4·yr^{-1} are respectively adsorbed on the flocculating iron hydroxide colloids and desorbed from marsh deposits. Marsh macrophytes are the main P consumers in the estuary (~100 t·yr^{-1}), but detectable PO_4 changes attributable to this biological uptake are confined to the areas near the marshes.

On présente un bilan du premier ordre pour le phosphore (P) de l'estuaire moyen du Saint-Laurent. La remontée d'eau profonde du Chenal Laurentien apporte 90% du P dissous dans l'estuaire, alors que les eaux douces du fleuve y charrient la plupart du P particulaire. Le flux du P dissous dans l'estuaire (~ 80 000 t·an^{-1}) est 10 fois plus important que celui du P particulaire (~ 5 000 t·an^{-1}, dont plus de la moitié sous forme d'apatite détritique). Néanmoins, les réservoirs de P dissous et particulaire sont du même ordre de grandeur (~ 3 000 t), en raison du court temps de résidence des masses d'eau par rapport à celui des solides en suspension. Ce rapide renouvellement des masses d'eau est à l'origine du comportement conservatif des orthophosphates dissous (PO_4) dans tout l'estuaire, apparemment non affecté par les échanges bio-géochimiques du P avec la matière particulaire. Environ 500 t·an^{-1} de PO_4 sont adsorbés sur les hydroxy-colloïdes de fer dans la zone de floculation et 300 t·an^{-1} désorbés depuis les dépôts récents sur les marais. Les macrophytes des marais sont les principaux utilisateurs de PO_4 dans l'estuaire (~ 100 t·an^{-1}). On ne peut détecter une consommation biologique de PO_4 que dans les zones contigües à ces marais.

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The study of phosphorus (P) as a life-sustaining nutrient has received considerable attention for many years. At least in some estuarine ecosystems, total biological productivity is limited by the concentration of available P. In an estuary, the reactive P is found in both dissolved and particulate forms. Both forms are highly interactive depending on changes in the geochemical environment and on biological utilization (Froelich et al. 1982; Sharp et al. 1984; Strom and Biggs 1982; Liss 1976; Lyons et al. 1980; Morris et al. 1981; Kemp et al. 1984; Carpenter and Smith 1984). Uncertainties regarding the adsorption of P on solid matter and the biological uptake of this element contribute to difficulties in estimating the world fluvial flux of reactive P to the oceans (Froelich et al. 1982).

Previous work has shown that dissolved orthophosphate (PO_4) displays a positive linear correlation with salinity throughout the St. Lawrence Upper Estuary (Lucotte and d'Anglejan 1983, 1988b). This relationship is contrary to the negative one found in most estuaries (Liss 1976). It indicates that P-rich intermediate waters from the lower estuary upwelling near the head of the Laurentian Channel (Fig. 1) are introduced by tides into the upper estuary (Greisman and Ingram 1977). There is no systematic departure from simple dilution to indicate that P is removed in the upper estuary as a result of biological consumption (Lucotte and d'Anglejan 1988b). Biological uptake is hindered by unstable physical conditions and high levels of turbidity which limit phytoplankton production (Cardinal and Bérard-Therriault 1976; Bah and Legendre 1985). PO_4 depletion is nevertheless detectable during the growing season in areas adjacent to the large intertidal marshes situated along the shores of the estuary (Fig. 1) (Lucotte and d'Anglejan 1988b). Macrophytes growing on the marshes may be responsible for this uptake; under similarly turbid coastal conditions, macrophytes are the major producers of photosynthetically fixed carbon (Gordon et al. 1985; Pomeroy et al. 1981).

Although the total suspended matter load in the St. Lawrence is low relative to other estuaries, the maximum turbidity zone (Fig. 1) represents a major reservoir in the estuarine P budget, with an average total P content of about 50 μg-at·g^{-1} (Lucotte and d'Anglejan 1983, 1988b). Particulate P reaches equilibrium values in the early salt mixing zone ($S < 1‰$), being coprecipitated there with flocculating iron hydroxide colloids. Partial P desorption occurs within the suboxic deposits formed seasonally on the intertidal flats of the Cap Tourmente region (Lucotte and d'Anglejan 1986, 1988a, 1988b). Sediments deposited on the marshes during the summer are eroded in the fall by the digging action of over 200 000 migratory greater

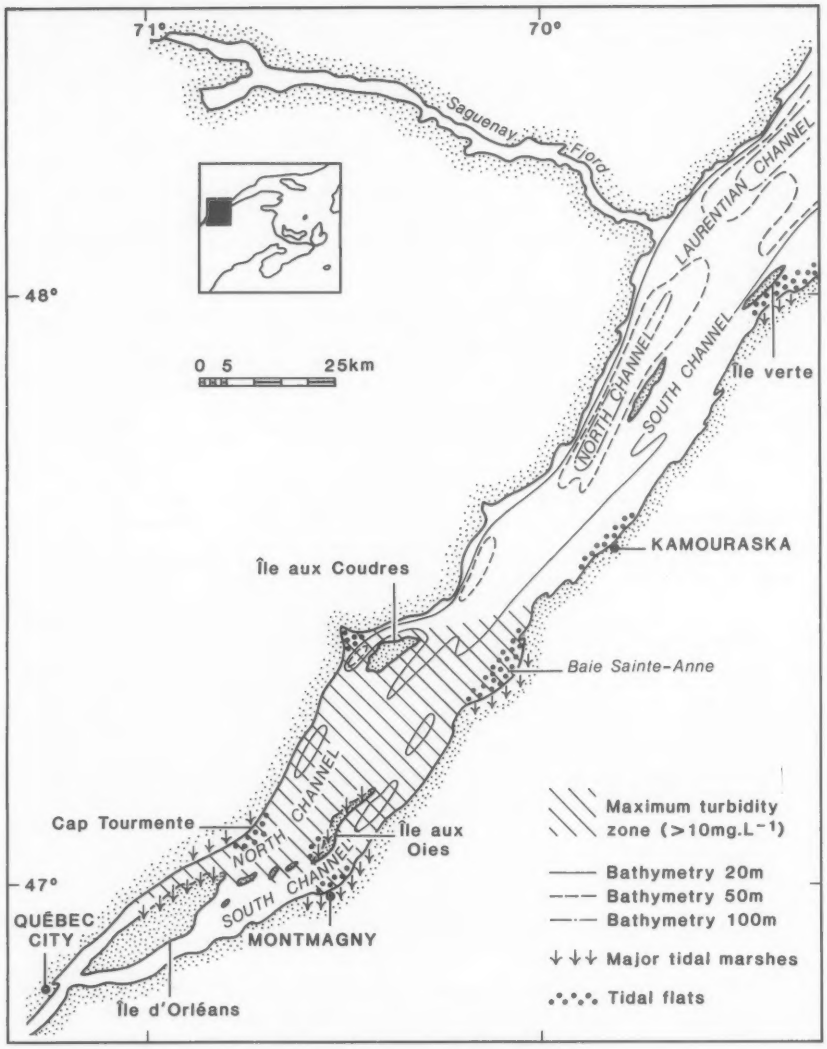

FIG. 1. St. Lawrence Upper Estuary.

snow geese (*Anser caerulescens atlantica*) probing for *Scirpus americanus* rhizomes. This onshore–offshore sediment cycle controls the release of P-depleted suspended matter to the estuary.

This paper combines new data with information from the literature to estimate a first-order P budget for the St. Lawrence Upper Estuary. The quantities of P involved in the different estuarine geochemical or biological processes are calculated and compared with the sizes of dissolved and particulate P reservoirs. The poor correlations between the dissolved and particulate P reservoirs and the corresponding adsorption–desorption reactions are looked upon in the light of the P fluxes and residence times.

Materials and Methods

Sampling

The results presented in this paper are a synthesis of the results obtained for the P–Fe study of the St. Lawrence Upper Estuary; data were collected during eight cruises at 23 stations from both the north and south channels of the estuary from 1981 to 1985 (Lucotte and d'Anglejan 1983, 1988b). The sampling periods were chosen to coincide with the spring runoff, the lowest summer freshwater discharge, and the presence of the migratory greater snow geese on the marshes in the fall. The currents, turbidity distributions, and sediment transport were described in a previous paper (Lucotte and d'Anglejan 1986). Suspended particulate matter was collected by continuous-flow centrifugation and rapidly freeze-dried. The detailed sample processing is described in Lucotte and d'Anglejan (1988b). The Cap Tourmente and Baie de Sainte-Anne tidal flats were also surveyed on foot during low tide in 1983 and 1984. Vegetation diversity, density, and height, as well as the thickness of freshly deposited muds, were measured and sediment samples taken. The plants were thoroughly washed, and subsequently dried at 60°C. The dry roots, stems, and leaves were then separately crushed for their respective determination of P content.

TABLE 1. Average dissolved and solid fluxes through the St. Lawrence Upper Estuary. n.a. = not applicable.

	Water fluxes ($m^3 \cdot s^{-1}$)	Water volume (m^3)	Solid fluxes ($t \cdot yr^{-1}$)	Solid content (t)
St. Lawrence River at Quebec City	11×10^3 [a]	n.a.	4×10^6 [d,e]	n.a.
Saline counter current	50×10^3 [b]	n.a.	Negligible	n.a.
Mixed outflow	61×10^3 [a,b]	n.a.	4×10^6 [e]	n.a.
Average for the St. Lawrence Upper Estuary	n.a.	7×10^{10} [c]	n.a.	2×10^6 [f]
Cap Tourmente tidal flats	n.a.	n.a.	2.2×10^5 [a,d]	n.a.
All flats together	n.a.	n.a.	$3-4 \times 10^6$ [d]	n.a.

[a]Lucotte and d'Anglejan 1986.
[b]Bewers and Yeats 1978.
[c]Godin 1971.
[d]This study.
[e]Dionne 1984.
[f]d'Anglejan et al. 1981.

Laboratory Analyses

Concentrations of dissolved P were measured with a Technicon II autoanalyser and regularly compared with the values obtained on the same samples by colorimetry (Lucotte and d'Anglejan 1983). Concentrations of dissolved organic P (P_{org}) were calculated by subtracting the PO_4 content from the total dissolved P (following a 2-h ultraviolet oxidation of the water sample) (Lucotte and d'Anglejan 1983).

Concentrations of particulate exchangeable P (P_{Ex}), particulate orthophosphate (P_{CDB}), P_{Org}, and apatite P (P_{Ap}) were measured by the citrate–dithionite–bicarbonate (CDB) extraction sequence. The CDB scheme was chosen for both its specificity and reproducibility (Lucotte and d'Anglejan 1983). The precision of the method is better than ±7% (95% confidence level) at all steps.

Results

Table 1 summarizes measured dissolved and solid fluxes for the St. Lawrence Upper Estuary. The suspended particulate matter flux entering the estuary from the St. Lawrence River was calculated using its monthly average freshwater discharges (Gouvernement du Québec, unpubl. data) and our estimates of suspended matter concentrations at Quebec City. The flux of 4×10^6 $t \cdot yr^{-1}$ (dry weight) is comparable with Dionne's (1984) estimate of 8×10^6 to 10×10^6 $t \cdot yr^{-1}$ (wet weight). The solid matter input to the estuary via the saline counter current is considered to be negligible relative to the freshwater load (d'Anglejan 1981). Over 40% of the annual suspended sediment input to the estuary occurs during the spring freshet (April–May).

The export of suspended particulate matter from the estuary must equal the river influx (Table 1), since no significant zones of fine sediment erosion or deposition are known either in the channels, along the shallow subtidal platforms of the south shore, or on the shorelines (d'Anglejan and Brisebois 1978; Sérodes et al. 1983; Dionne 1984). Moreover, the annual sedimentary budget on the tidal marshes is in balance with respect to supply and loss, in spite of important deposition in the summer (see below) and local erosion of the upper marshes (Dionne 1986).

A massive deposition of suspended flocs occurs within the 1.75×10^6 m^2 vegetated area of the intertidal flats of Cap Tourmente (Fig. 1) during the summer months (Lucotte and d'Anglejan 1986). The average accumulation reaches 10 cm by September before the erosion by the geese (Sérodes and Troude 1984; Dionne 1985). The deposits having a mean density of 2.5 $g \cdot cm^{-3}$ and a 50% water content, the temporary annual sedimentary storage is $\sim 2.2 \times 10^5$ t (dry weight) (Table 1).

With the inclusion of the tidal marshes of the Montmagny region (Fig. 1), the total surface area similarly colonized by *Scirpus americanus* is $\sim 39 \times 10^6$ m^2 (Dryade 1980). Close to the core of the maximum turbidity zone, centered around Cap Tourmente, these marshes are also buried with up to 8–10 cm of new sediments by September. This brings the total amount of solid matter annually deposited in the upstream region of the estuary to a minimum of 3×10^6 $t \cdot yr^{-1}$. Downstream, *Spartina alterniflora* marshes between Kamouraska and Île Verte (Fig. 1) cover an area of about 14×10^6 m^2 (Dryade 1980). The annual sediment deposition, before erosion in the fall, does not exceed 2 cm (d'Anglejan et al. 1981; Sérodes et al. 1983). This represents an additional 3.5×10^5 t (dry weight) of particulate matter involved in the yearly sedimentary cycle on the marshes. Our overall estimate of 3×10^6 to 4×10^6 $t \cdot yr^{-1}$ for marsh and tidal flat deposition in the St. Lawrence Upper Estuary (Table 1) is comparable with that of Sérodes (1980).

The average suspended particulate matter content in the maximum turbidity zone is $\sim 2 \times 10^6$ t (Table 1) (Forrester 1972; d'Anglejan et al. 1981). This estimate is only an approximation, since a precise determination of the suspended matter distribution is made difficult by the large tidal and seasonal fluctuations in concentrations (Lucotte and d'Anglejan 1986). It is probably much higher both during the spring freshet and the fall erosion of the marshes when high solid matter fluxes are introduced to the turbid zone.

Table 2 gives the annual ranges in concentration of dissolved PO_4 and of dissolved P_{org} for both the freshwater and the saltwater inputs, as reported by Lucotte and d'Anglejan (1983,

TABLE 2. Dissolved and particulate P ranges of concentrations, followed by their annual average and standard deviation (in parentheses) (Lucotte and d'Anglejan 1983, 1988b). n.a. = not applicable.

	Freshwater inputs	Saline countercurrent	Estuarine average	Cap Tourmente tidal flats (October)
Dissolved P ($\mu g\text{-at}\cdot L^{-1}$)				
PO_4	0.35 – 0.57 0.42 (0.08)	1.08 – 1.42 1.25 (0.14)	1.10	n.a.
P_{org}	0.19 – 0.61 0.40 (0.25)	0.06 – 0.23 0.14 (0.09)	0.19	n.a.
Particulate P ($\mu g\text{-at}\cdot g^{-1}$)				
$P_{CDB} + P_{Ex}$	9.7 – 12.0 10.5 (1.1)	n.a.	11.2 – 16.2 14.5 (1.6)	10.7 – 12.0 11.3 (0.6)
P_{Org}	5.8 – 6.5 6.2 (0.3)	n.a.	2.2 – 6.3 4.5 (1.4)	3.9 – 6.1 5.2 (1.0)
P_{Ap}	23.8 – 24.9 24.4 (0.6)	n.a.	22.9 – 26.4 24.1 (1.4)	22.7 – 25.2 24.2 (1.1)

TABLE 3. Maximum standing crop (mid-September) and fall regeneration of the three major macrophytes growing on Cap Tourmente tidal marshes (dry weights) and associated P contents.

	Maximum standing crop (Sept.) (t)[a]	Debris regenerated in the fall (t)[a]	$P_{CDB} + P_{Ex}$ ($\mu g\text{-at}\cdot g^{-1}$)	P_{Org} ($\mu g\text{-at}\cdot g^{-1}$)
Scirpus americanus				
Green stems	293	293	102.1	10.3
White shoots	30	30		
Surface rhizomes	70	45	147.1	16.8
Deep rhizomes	286	0		
Sagittaria latifolia				
Leaves	33	33	54.2	15.3
Green stems			104.7	8.2
White shoots	38	30		
Roots	42	0	217.5	22.1
Zizania aquatica				
Green stems	263	263	110	
Roots	42	42	160	

[a]Reed (1984).

1988b). The corresponding annual averages were obtained using our data taken from 1983 to 1985. They agree with the observations of Takayanagi and Gobeil (1985). The mean dissolved concentration for the estuary as a whole was calculated on the basis of the previous average values applied to the respective fresh and saline fluxes of Table 1.

The partitioning of particulate P (Table 2) is given for three distinct environments used to group the results obtained on 140 estuarine seston samples (Lucotte and d'Anglejan 1988b): freshwater input, estuarine average, and tidal flats.

The maximum standing crops of macrophytes growing on the Cap Tourmente marshes in 1979 (Table 3) were evaluated by Reed (1984) by means of in situ counts of stems and associated rhizomes on selected transects. These estimates are in agreement with other values found in the literature (Doran 1978; Reed 1978; Sérodes and Troude 1984; Smith and Odum 1981) and are used for budget calculations in this paper. An estimate of the plant biomass exported from the marsh to the estuary in the fall is based on the foraging of the migrating geese for their favorite food, *Scirpus americanus* white shoots and surface rhizomes. On average, two thirds of the *Scirpus* rhizomes over the marsh surface are used by the birds (Reed 1984; Doran 1978). At the same time, all *Zizania aquatica* and the aerial parts of *Sagittaria latifolia* are also returned to the open estuarine waters. Table 3 gives the various P fractions obtained on different parts of the plants.

A first-order P budget for the St. Lawrence Upper Estuary (Table 4) has been calculated by using all available data and by extrapolating the Cap Tourmente results to the entire estuary as follows. Estimates of the dissolved P inputs from the St. Law-

TABLE 4. Annual P budget in the St. Lawrence Upper Estuary.

Dissolved P (t)		Particulate P(t)	
	Annual inputs		
PO_4	4 800[a]	$P_{Ex} + P_{CDB}$	1220[a]
	+65 000[b]	P_{Org}	650[a]
P_{org}	4 600[a]	P_{Ap}	3030[a]
	+7 300[b]		
	Reservoirs		
PO_4	2 400	$P_{Ex} + P_{CDB}$	900
P_{org}	400	P_{Org}	280
		P_{Ap}	1500
	Flocculation		
	500	$P_{Ex} + P_{CDB}$	500
	Sedimentary cycle		
Desorption of anoxic muds	300 – 400	Temporary storage $P_{Ex} + P_{CDB}$	1350
		P_{Org}	420
		P_{Ap}	2240
	Macrophytes		
Annual consumption and regeneration	80	Maximum standing crop $P_{Ex} + P_{CDB} + P_{Org}$	140
	Annual seaward flux		
	~81 500		~5100

[a]Freshwater inputs.
[b]Saltwater countercurrent.

rence River, based either on average annual discharge and concentrations (Tables 1 and 2) or on monthly values obtained in 1984 for both freshwater runoff (Gouvernement du Québec, unpubl. data) and PO_4 concentrations (Takayanagi and Gobeil 1985), are in agreement. A major uncertainty concerning the P inputs from the saline countercurrent lies in the difficulty in evaluating the volume of water introduced per tide at the head of the Laurentian Channel. Although the PO_4 concentration of the deepwater mass is fairly constant all year round, the rate of upwelling varies considerably throughout time, from neap to spring tides, for example (Muir 1979). For particulate P, the input is mainly from the river end (Table 1) and shows a characteristic partitioning between the various fractions (Table 2). The particulate P flux to the estuary (Table 4) may therefore be estimated by multiplying the freshwater solid flux by the corresponding P concentrations.

The dissolved P reservoirs (Table 4) have been determined as the product of the mean PO_4 and P_{org} concentrations by the estuarine volume (Tables 1 and 2). The particulate P reservoir is estimated using the average P fractions and the suspended particulate matter content of the turbid zone.

The 500 t of P removed by flocculation (Table 4) is calculated using the difference between the mean freshwater reactive P ($P_{Ex} + P_{CDB}$) content (10.5 $\mu g\text{-at}\cdot g^{-1}$) and that of the estuarine average (14.5 $\mu g\text{-at}\cdot g^{-1}$) applied to the annual freshwater suspended particulate matter load to the estuary. The 300–400 t of P desorbed on the marshes is estimated by the difference between the average reactive P content of the turbidity zone and that of the tidal flats (11.3 $\mu g\text{-at}\cdot g^{-1}$) applied to the annual sedimentary budget on the marshes.

The estimates (Table 4) of P held and regenerated by the macrophytes in the whole estuary were obtained by extrapolating the Cap Tourmente results of Table 3 to the marsh area of the Montmagny region (Table 5). In addition, one must include the contribution of the downstream *Spartina* marshes, covering 14×10^6 m^2 (Dryade 1980): the maximum standing crop was calculated using the 400 $g\cdot m^{-2}\cdot yr^{-1}$ aerial production (Sérodes et al. 1983) and a below-ground to above-ground ratio of 0.5–0.7 (Marinucci 1982). This represents about 35 additional t of P retained by the plants, more than half of it being returned to the estuary in the fall (Table 5). The P retention by the plants was calculated without taking into consideration the P turnover by the plant roots or the bacteria. The annual P out-

TABLE 5. Macrophytes maximum standing crops and fall regeneration in the St. Lawrence Upper Estuary and the P involved.

	Maximum standing crop (Sept.) (10^3 t)	Debris regenerated in the fall (10^3 t)	P maximum standing crop (t)	P regeneration (t)
Scirpus americanus (Cap Tourmente)	0.7[a]	0.4[a]	2.9	1.4
Scirpus americanus (estuary)	15	8	65	30
Sagittaria latifolia (Cap Tourmente)	0.1[a]	0.06[a]	0.7	0.3
Sagittaria latifolia (estuary)	2.5	1.4	15	7
Zizania aquatica (Cap Tourmente)	0.3[a]	0.3[a]	1.1	1.1
Zizania aquatica (estuary)	6.8	6.8	25	25
Total (*Scirpus* marshes)	24	16	105	62
Spartina alterniflora marshes (total)	9.0	5.6	36	19
Total	33	22	141	82

[a]Reed (1984).

puts given in Table 4 were calculated considering that the St. Lawrence Upper Estuary does not act as a source or as a trap for sediments. Consequently, the annual dissolved and particulate P seaward fluxes equal the annual inputs after having taken into consideration their exchange reactions.

Discussion and Conclusions

Although this work relies on still tentative estuarine fluxes, it constitutes a first-order P budget allowing the comparison between the dissolved and particulate reservoirs and their mutual transfers. The dissolved P introduced to the estuary each year is mainly upwelled from the intermediate waters of the Laurentian Trough. This dissolved P flux exceeds by a factor of 10 the particulate flux. However, their respective instantaneous reservoirs are of the same order of magnitude because of the short residence time of the water masses relative to that of the particulate matter. Based on the flux values given in Table 1 and the estuarine volume, the mean water residence time in the upper estuary is of the order of 10–20 d. This figure is in agreement with the measured flushing time for the spring freshet of 1 mo between Quebec City and the lower estuary (El Sabh 1979). In contrast, a mean residence time of at least 1 yr for the estuarine particulate matter can be estimated: i.e. the yearly river solid flux is approximately equal to the sum of the suspended particulate matter in the turbidity maximum zone and the fine sediments temporarily deposited on the marshes (Table 1). These estimates agree with those of d'Anglejan and Smith (1973) and Dionne (1984) who stated that most of the estuarine solid matter inputs remain in the maximum turbidity zone until they are incorporated in the ice-cover in winter and drift away upon breakup. Consequently, because of rapid turnover of the water masses, measurements of dissolved species in the estuary will miss the effects of biogeochemical exchange reactions with particles in suspension. This explains the general conservative behaviour of dissolved PO_4 throughout the estuary.

PO_4 adsorption on iron hydroxides colloids in the mixing zone is the major process for the transfer from the dissolved to the particulate P reservoirs. The value of 500 t for this uptake increases the reactive particulate P ($P_{Ex} + P_{CDB}$) flux annually carried by the river by about 50% (Table 4). However, the effects of the reaction are hardly felt on ambient PO_4 concentrations. Only the situation prevailing in the early mixing zone at the upstream end of the South Channel indicates significant PO_4 departures (by up to 75%) from the theoretical dilution lines (Lucotte and d'Anglejan 1988b). The dilution is limited by reduced water transport through the narrow channel. Also, Fe flocculation and P coprecipitation occur mainly in the salinity range from 0 to 1‰. Finally, P uptake is only noticeable in periods of highest particulate organic matter content (May 1985 and October 1984), an essential requirement for the precipitation of the Fe colloids (Sharp et al. 1984). Indeed, values of adsorbed P higher than those reached at equilibrium have been reported in this region, compared with the maximum turbidity zone further downstream (Lucotte and d'Anglejan 1988b). In contrast, in similarly low brackish zones of the north channel which are influenced by tidal exchanges with the Cap Tourmente marshes, no significant P adsorption above the equilibrium value or PO_4 uptake could be detected. This suggests that spontaneous Fe flocculation only occurs over the south channel, the main route for the St. Lawrence River outflow.

The total of 300 t for desorbed P from the recently deposited sediments on the tidal marshes is of the same order of magnitude as the quantities of P adsorbed, as discussed above (Table 4). Again, while this release is equivalent to at least 30% of the total reservoir of reactive P ($P_{Ex} + P_{CDB}$) in the estuary, most of the additional PO_4 is rapidly diluted by mixing in the estuary. In August 1984, representative of summer conditions before the sediment erosion starts on the flats, the average dissolved PO_4 concentrations are about 25% higher than the theoretical dilution line in the early mixing zone along the margin of the large marshes (Lucotte and d'Anglejan 1988b). This is interpreted as reflecting the slow but massive upward diffusion of the PO_4 released in the suboxic layers of the flats, a process which affects PO_4 values only locally. In the fall (October 1983 and 1984), upward diffusion of PO_4 slows down as the surface sediments are being quickly oxidized by bird foraging and wave action. Significant excesses in PO_4 can be detected only for 2 h near low tide off the marshes. This is the time when dilution by mixing is minimal with sluggish currents, low water levels, and a flow direction away from the marshes.

Once the P-depleted solids are returned from the marshes to the channels in the fall, P readsorption appears to be fairly slow and is not completed until the following spring (Lucotte and d'Anglejan 1988b). No detectable PO_4 uptake is noticed during reequilibration.

The growth of macrophytes is the major utilization of P in the marshes. The importance of other biological uptake such as by benthic diatoms is unknown, but might be significant (Marinucci 1982). The estimated 100 t of P involved in the annual marsh plant cycle is small when compared with the adsorption–desorption reactions (Table 4). Consumption of PO_4 on the marshes is only measurable during the growth season (May 1985, June 1984, August 1984), when PO_4 uptake occurs near low slack tide off the marshes; at that time, mixing of ebbing water with the estuary is minimal (Lucotte and d'Anglejan 1988b). When the dead plant debris is returned to the estuary, the potential PO_4 regenerated by bacteria (Painchaud et al. 1987) is undetectable. This again is masked by large dilution effects and, perhaps, by slight incipient readsorption of P on P-depleted solids which are returned to the water.

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Genetic Variation in Steelhead (*Salmo gairdneri*) from the North Coast of Washington

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Reisenbichler, R. R., and S. R. Phelps. 1989. Genetic variation in steelhead (*Salmo gairdneri*) from the north coast of Washington. Can. J. Fish. Aquat. Sci. 46: 66–73.

Steelhead (*Salmo gairdneri*) collected from various sites in nine drainages in northwestern Washington were genetically characterized at 65 protein-coding loci by starch-gel electrophoresis. Genetic differentiation within and among drainages was not significant, and genetic variation among drainages was much less than that reported in British Columbia; these results may be the consequence of gene flow from hatchery stocks that have been released in Washington since the 1940's. Allele frequencies varied significantly among year-classes (hence, genetic characterization studies must include data from several year-classes), and also between hatchery fish (including a stock developed with local wild fish) and wild fish, indicating that few wild fish have been successfully and routinely included in hatchery brood stocks. Conservation of genetic diversity along the north coast of Washington should be facilitated by reducing the numbers of hatchery fish that spawn in streams and by including wild fish in hatchery brood stocks.

Des truites arc-en-ciel (*Salmo gairdneri*) prélevées en divers points de neuf bassins versants du nord-ouest de l'état de Washington ont fait l'objet d'une caractérisation génétique par électrophorèse sur gel d'amidon portant sur 65 locus de codage des protéines. La différenciation génétique au sein ou entre les bassins n'était pas significative et la variation génétique entre les bassins était beaucoup moins importante que celle signalée en Colombie-Britannique. Ces résultats peuvent s'expliquer par un apport génétique à partir des stocks d'élevage qui ont été ensemencés dans l'état de Washington depuis les années 1940. Les fréquences des allèles variaient de façon significative entre les classes annuelles (la caractérisation génétique doit donc porter sur les données de plusieurs classes annuelles) et entre les poissons d'élevage (y compris un stock produit à partir de poissons sauvages locaux) et les poissons sauvages : très peu de poissons sauvages ont donc été intégrés avec succès et de façon constante aux stocks de géniteurs d'élevage. Le maintien de la diversité génétique le long de la côte de l'état de Washington pourrait être facilité par la réduction du nombre de poissons d'élevage frayant dans les cours d'eau et par l'inclusion de poissons sauvages aux stocks de géniteurs d'élevage.

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Steelhead (*Salmo gairdneri*) are distributed from central California to the Alaska Peninsula (Sutherland 1973). The fish hatch in freshwater streams where they remain for 1–4 yr (generally 2 or 3 yr) and then migrate to sea where they live for up to 4 yr before returning to the freshwater streams (generally their natal streams) to spawn (Shapovalov and Taft 1954; Withler 1966). Because homing to the natal stream limits gene flow, populations (stocks) in different streams can differentiate genetically (Parkinson 1984).

State and federal fisheries management agencies are charged with the conservation and management of fisheries resources, and this charge requires protection of the associated genetic characteristics (Altukhov 1981; Frankel 1983): the specific genes, gene combinations, and gene pools of genetically differentiated populations. Although genetic differentiation is recognized as an important factor in fisheries management (Simon and Larkin 1972; Helle 1981), few data are available for describing the degree of differentiation (i.e. the genetic structure) in most fish species.

Utter et al. (1980) identified two genetically distinct groups of steelhead (coastal and inland) along the west coast of North America, and Parkinson (1984) showed genetic differentiation among populations of steelhead from adjacent streams over a broad portion of British Columbia. The present study was initiated to genetically characterize the populations of steelhead from the major drainages of the north coast of Washington: the Quillayute, Hoh, Queets, and Quinault rivers and the northern portion of the Chehalis Basin (Fig. 1). These streams adjoin or are partly contained in Olympic National Park and were selected because the United States Congress has directed that the natural resources of national parks be conserved. There is also international concern for conservation of natural (including genetic) resources in the park, as indicated by inclusion of the park in the International Biosphere Reserve Program (Franklin 1977). Steelhead from two other streams (Skagit River and Snow Creek) and two other hatcheries (Elwha Hatchery and South Tacoma Hatchery; Fig. 1) in northwestern Washington were also sampled to enhance our perspective for examining north coast fish. Starch-gel electrophoresis was used to genetically characterize the fish.

This study was to investigate the genetic structure of wild populations by testing for genetically distinct populations within

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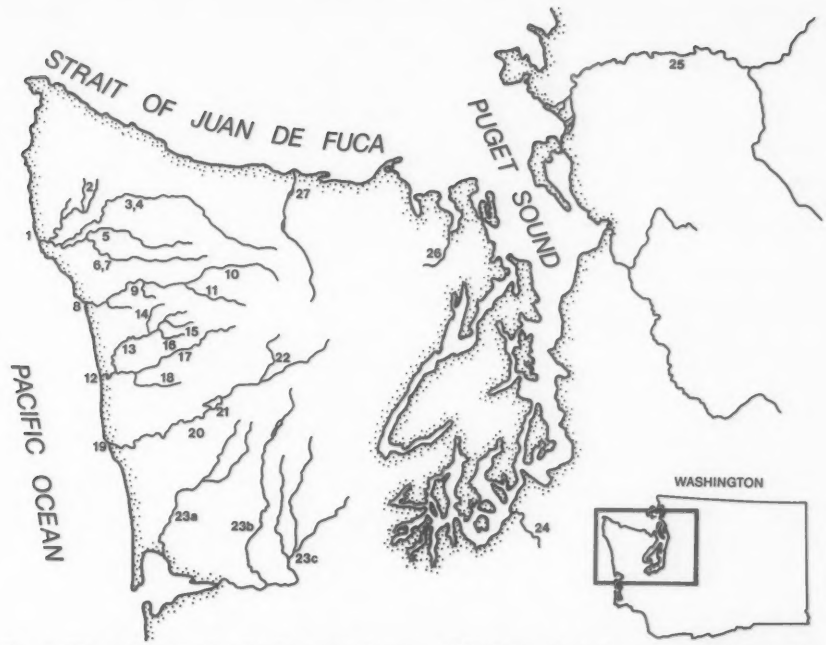

FIG. 1. This study focused on the five major drainages of Washington's north coast: the Quillayute (1), Hoh (8), Queets (12), Quinault (19), and northern Chehalis (23). Numbers identify sampling areas: (1) Quillayute River (nets); (2) Dickey River; (3, 4) Soleduck River; (5) Calawah River; (6) Bogachiel River; (7) Bogachiel Ponds; (8) Hoh River (nets); (9) lower Hoh River, i.e. below South Fork; (10) upper Hoh River, i.e. above South Fork; (11) South Fork Hoh River; (12) Queets River (nets); (13) mainstem Clearwater River; (14) Snahapish River; (15) Solleks River; (16) Stequalaho Creek; (17) Queets River above Salmon River; (18) Salmon River; (19) Quinault River (nets); (20) Quinault National Fish Hatchery (Quinault NFH); (21) Quinault Tribal Rearing Facility (Quinault Pens); (22) upper Quinault River, i.e. above Lake Quinault; (23) northern Chehalis Basin; (24) South Tacoma Hatchery; (25) Skagit River; (26) Snow Creek; (27) Elwha Hatchery. (Nets = adults were taken in the Indian net fisheries.)

TABLE 1. Origin of hatchery stocks.

Stock	Run	Origin
Bogachiel Rearing Ponds (or Bogachiel Ponds)	Winter	South Tacoma Hatchery (southern Puget Sound)
Elwha Hatchery	Winter	South Tacoma Hatchery
Quinault National Fish Hatchery (or Quinault NFH)	Winter	Quinault River
Quinault Tribal Rearing Facility (or Quinault Pens)	Winter	Quinault NFH and Quinault and Queets rivers
Soleduck River	Summer	Skamania Hatchery (Columbia River)

and between drainages and to genetically compare hatchery and wild (i.e. naturally spawned) fish. Although hatchery fish have been shown to differ genetically from wild fish in traits affecting survival (Reisenbichler and McIntyre 1977; Chilcote et al. 1986), they may not differ in allozyme frequencies if, for example, wild fish are routinely included as brood fish in the hatchery.

Comparison of our results with those of Parkinson (1984) was of special interest because the steelhead populations examined by Parkinson were nearly (genetically) pristine: most hatchery programs for steelhead in British Columbia were started after 1976, primarily with wild fish from the local area to develop the hatchery stock (D. Narver, British Columbia Fish and Wildlife Branch, 780 Blanschard St., Victoria, B.C. V8V 1X5, pers. comm.). In contrast, hatchery fish have been released into north coast drainages in Washington since the 1940's and most of these fish were derived from one hatchery population, the South Tacoma stock from southern Puget Sound (J. Nielson, Washington Department of Wildlife, 600 N. Capitol Way, Olympia, WA, pers. comm.). Hatchery fish, through interbreeding with wild fish, can genetically alter wild (naturally spawning) populations and thereby compromise genetic

conservation (Reisenbichler and McIntyre 1977; Helle 1981; Reisenbichler 1984; Nelson and Soulé 1987).

Materials and Methods

Two runs of steelhead, distinguished by the time of year when they return to freshwater as adults, occur in the study area. Winter-run fish (by far the more abundant run) return to freshwater from November to April, and most summer-run fish return from May to July. Most of the summer-run fish (adults) caught in north coast streams are hatchery fish (S. Chitwood, Quileute Tribal Fisheries Department, P.O. Box 279, La Push, WA 98350, pers. comm.; L. Lestelle, Quinault Tribal Fisheries Department, P.O. Box 189, Taholah, WA 98587, pers. comm.), so summer-run adults were not included in the analyses of genetic variation (described below). Chilcote et al. (1980) found no significant differences in isoallele frequencies, relative to variation among year-classes, between summer-run and winter-run steelhead from the Kalama River, Washington, and similar results have been obtained from the north coast of Washington and from the Oregon coast (U.S. Fish and Wildlife Service, unpubl. data).

Sample Collection

Fish were collected, primarily during the spring and summer of 1983, from the 27 areas identified in Fig. 1. Juvenile fish at hatcheries (Table 1) were collected with dip nets at several locations around each raceway containing the fish to be studied. Juveniles in streams were captured by trapping, electrofishing, and seining. A few juveniles (usually less than 15 of each age) were taken from each of several sites throughout each drainage, except that areas where 1982 brood hatchery fish had been released were not sampled. Juveniles were kept alive or held on ice for up to 8 h and then frozen at −10 or −70°C until they were thawed for electrophoretic analysis.

Samples of tissue from eye, liver, white muscle, and heart were taken from adult fish spawned at hatcheries or caught in gillnet fisheries at the mouths of the rivers as described by Reisenbichler and Phelps (1987). Many of the winter-run adults harvested in the net fisheries were hatchery fish; however, most winter-run hatchery fish were harvested by these fisheries before February (Quileute Fisheries Department 1983), and most fish taken after 1 February were wild fish (S. Chitwood, Quileute Tribal Fisheries Department, P.O. Box 279, La Push, WA 98350, pers. comm.). We sampled only winter-run fish that were delivered to fish buyers after 1 February, and among these, fish with fewer than two freshwater annuli on their scales were considered to be hatchery fish and were excluded from the analysis.

In most streams, juveniles exhibited a well-defined bimodal size distribution and were assigned to the 1982 or 1983 year-class on the basis of their size. When the two modes were not distinct, fish were not assigned to a year-class and were not used for comparing year-classes (see analysis section).

Juveniles from the Chehalis Basin and the Skagit River were reared in hatcheries, but were the offspring of wild fish. The Chehalis Basin fish were a mixture of fish from the three northern streams of the Chehalis Basin, the Humptulips, Wynoochee, and Satsop rivers. The Skagit River fish and each of the year-classes of Chehalis Basin fish were the offspring of at least 60 males and 60 females.

Electrophoresis

We used horizontal starch-gel electrophoresis (Utter et al. 1974; May et al. 1979) to assay fish tissues. Eye, heart, liver, and muscle tissues were removed from partly thawed juvenile fish just before electrophoretic analysis. We identified alleles at loci encoding specific enzymes, using the staining methods of Harris and Hopkinson (1976) and Allendorf et al. (1977). The nomenclature used to describe the gene loci and the allele variants followed Allendorf and Utter (1979).

Of the 43 enzymes examined, 33 had sufficient activity and resolution for use in this study (Table 2). Initially all 33 enzymes, coded by 65 loci, were assayed in all fish; in later samples, however, we omitted the loci that had been determined to be monomorphic.

Fish with the "fast" allele at *Me-4* or *Sdh-1* or the 86 allele at *Idh-3,4* were considered to be cutthroat trout (*Salmo clarki*) or cutthroat–steelhead hybrids (Campton and Utter 1985); 21 fish that had one or more of these alleles were excluded from the analysis.

Statistical Analyses

The statistical procedures followed Reisenbichler and Phelps (1987). We used the chi-square test to examine genotype frequencies for deviation from the (Hardy–Weinberg) proportions

TABLE 2. Nine most variable enzymes of the 43 examined, and the associated loci.[a] Tissue E refers to eye, H to heart, L to liver, and M to white muscle. Buffer RW was described by Ridgeway et al. (1970) and AC by Clayton and Tretiak (1972).

Enzyme	Enzyme Commission No.	Locus designation	Tissue	Buffer
N-Acetyl-β-glucosaminidase	3.2.1.30	*bGa-1*	L	RW
Aconitate hydratase	4.2.1.3	*Ah-3*	L	AC
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3pdh-1*	M	AC
Isocitrate dehydrogenase	1.1.1.42	*Idh-2*	M	AC
		Idh-3,4	L	AC
L-Lactate dehydrogenase	1.1.1.27	*Ldh-4*	L, M, E	RW
Malate dehydrogenase	1.1.1.37	*Mdh-1,2*	L	AC
		Mdh-3,4	M	AC
Malate dehydrogenase (NADP+)	1.1.1.40	*MdhP-3*	M	AC
Phosphoglycerate kinase	2.7.2.3	*Pgk-2*	M	AC
Superoxide dismutase	1.15.1.1	*Sod-1*	L, M	RW

[a]Complete listing of enzymes and loci examined is available in Reisenbichler and Phelps (1985).

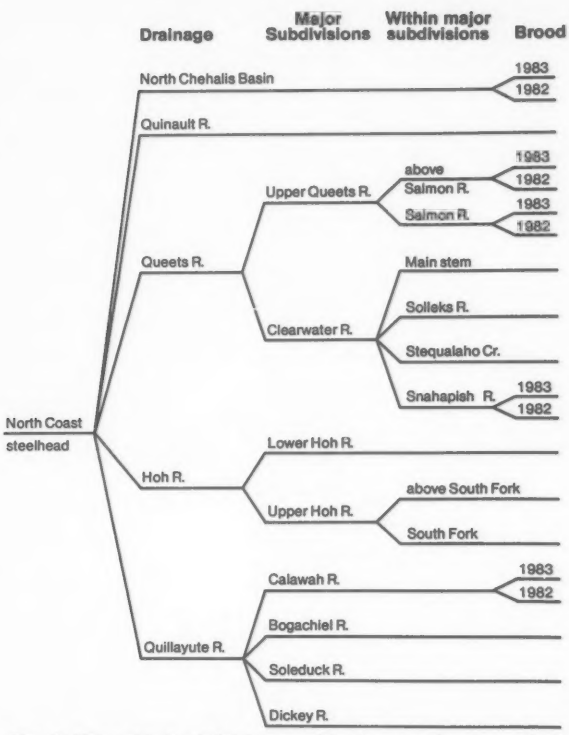

FIG. 2. Hierarchical subdivisions used in the gene diversity analysis for wild steelhead from the north coast of Washington. Brood (year-class) was not indicated where data for several broods were pooled.

expected with random mating and the likelihood ratio test (*G*-test; Sokal and Rohlf 1981) to test equality in allele frequencies between year-classes. The significance level for each test was modified to account for the increase in type I error when multiple tests of the same hypothesis are made (Cooper 1968). Tests were considered significant if the test statistic exceeded the critical value associated with a probability of $0.05/n$, where n was the number of loci tested within a sample (Hardy–Weinberg proportions) or the number of samples tested (variation between year-classes). Genotypes for *Idh-3,4*, *Mdh-1,2*, and *Mdh-3,4* were not tested because these systems consisted of pairs of loci with identical electrophoretic mobility, and genotypes at each locus could not be determined.

We used nested analysis of variance (ANOVA) to test intra-drainage differences in allele frequencies and one-way ANOVA to test differences between hatchery fish and wild fish (by contrasts; Snedecor and Cochran 1967; SPSS, Inc. 1983) and differences among drainages. The nested ANOVA consisted of three levels: year-classes within subdrainages (corresponding to the "major subdivisions" or, when present, the "within major subdivisions" levels in Fig. 2) within drainages. For the one-way ANOVA's, each drainage or hatchery occupied a cell with year-classes as replicates; winter-run adults were included as a year-class because they would have been the parents of the 1984 year-class. Comparisons of hatchery fish and wild fish were considered significant if the *F*-statistic exceeded the critical value associated with a probability $0.05/n$, where 0.05 was the desired probability of a type I error, and n was the number of loci used to test each contrast. The data were from the loci

TABLE 3. Likelihood ratio analysis of interbrood variation at 13 codominant loci of steelhead: *Ah-3*, *G3pdh-1*, *Idh-2*, *Idh-3,4*, *Ldh-4*, *Mdh-1,2*, *Mdh-3,4*, *MdhP-3*, *Pgk-2*, and *Sod-1*. Samples of hatchery fish are considered separately from samples of wild fish. Values for the Calawah River fish were not included in the total because the Calawah River is in the Quillayute River drainage (df = degrees of freedom, G = likelihood ratio statistic). $*P<0.05/s$, where $s = 8$ for wild fish and 4 for hatchery fish; $**P<0.01$.

	Total for all loci	
Samples	df	*G*
Wild fish		
Calawah River	10	15.76
Quillayute River	25	45.06
Hoh River	7	3.34
Snahapish River	8	23.34*
Salmon River	10	25.53*
Upper Queets River	10	21.27
Wynoochee River	8	17.17
Snow Creek	7	48.37*
Total for wild fish, excluding the Calawah River	75	184.08**
Hatchery fish		
Soleduck (summer run)	8	13.23
Bogachiel Ponds (winter run)	6	3.92
Quinault NFH (winter run)	7	30.11*
S. Tacoma Hatchery (winter run)	8	20.05*
Total for hatchery fish	29	67.31**

scored for fish from each major drainage and with frequencies less than 0.95 for the common (100) allele. The values used in the analyses were the arcsin of the square root of the frequencies of the common allele at each locus.

A modification of Chakraborty's (1980) gene diversity analysis was used to partition the total gene diversity of wild fish (H_t, heterozygosity of allele frequencies over locations) into hierarchical levels: $H_t = H_s + D_{bw} + D_{wm} + D_{md} + D_{dt}$, where H_s is the average heterozygosity within samples, D_{bw} is the gene diversity between broods, D_{wm} is the diversity within major subdivisions, D_{md} is the diversity among major subdivisions within drainages, and D_{dt} is the diversity among drainages (Nei 1973; Fig. 2). The modification consisted of giving equal weight to subgroups within a cell rather than weighting them according to the number of samples within each subgroup. Relative gene diversities (G_{ij}) were calculated as the proportion of the total gene diversity (H_t) due to a particular hierarchical level, for example, $G_{md} = D_{md}/H_t$. The gene diversity analysis, in contrast with the ANOVA, used the data from all loci and all alleles (Table 2).

The unweighted pair group method of cluster analysis (UPGM analysis; Sneath and Sokal 1973), applied to values of Nei's (1972) genetic distance calculated for each pair of samples, was used to illustrate genetic similarities among samples. Data from separate year-classes were pooled with equal weight for the cluster analysis.

Results

Fish from two locations showed significant deviation from Hardy–Weinberg proportions: the combined 1982 and 1983 broods from the Skagit River had an excess of heterozygotes at the *Pgk-2* locus, and the 1982 brood from Quinault National

TABLE 4. Allele frequencies for the 10 most variable loci, or pairs of loci (seven of which were used in the ANOVA (see Table 5)), for steelhead in northwestern Washington.[a] Each allele is designated by its mobility relative to the common allele × 100. *N* is the number of fish scored for most loci; however, fewer fish may have been scored at some loci. Frequencies from fewer than *N*/2 fish are identified with an asterisk, and frequencies from fewer than 25 fish were not presented or used in our analyses. SR = summer run.

		Ldh-4			*Mdh-3,4*				*Sod-1*			*MdhP-3*		*G3pdh-1*	
Location	*N*	100	76	40	100	75	83	120	100	152	38	100	88	−100	10
Wild fish															
Quillayute R.	494	0.946	0.054	0.000	0.879	0.087	0.027	0.007	0.620	0.377	0.002	0.860	0.140	0.981	0.019
Hoh R.	302	0.907	0.091	0.002	0.867	0.110	0.013	0.010	0.621	0.379	0.000	0.824	0.176	0.988	0.012
Queets R.	559	0.932	0.064	0.000	0.864	0.126	0.003	0.007	0.561	0.439	0.000	0.749	0.251	0.989	0.011
Upper Quinault R.	66	0.909	0.068	0.000	0.785	0.082	0.133	0.000	0.614	0.386	0.000	0.841	0.159	0.977	0.023
N. Chehalis Basin	240	0.939	0.061	0.000	0.849	0.146	0.005	0.000	0.686	0.305	0.009	0.734	0.266	0.934	0.066
Skagit R.	61	0.942	0.058	0.000	0.885	0.078	0.029	0.008	0.619	0.381	0.000	0.869	0.131	1.000	0.000
Snow Cr.	84	0.935	0.065	0.000	0.917	0.066	0.012	0.005	0.542	0.458	0.000	0.841	0.159	0.994	0.006
Hatchery fish															
Soleduck (SR)	80	0.844	0.156	0.000	0.927	0.073	0.000	0.000	0.719	0.281	0.000	0.919	0.081	0.885	0.115
Bogachiel Ponds	79	0.851	0.149	0.000	0.921	0.070	0.006	0.003	0.589	0.411	0.000	0.911	0.089	0.988	0.012
Quinault NFH	80	0.756	0.244	0.000	0.880	0.114	0.003	0.003	0.637	0.363	0.000	0.950	0.050	0.988	0.012
Quinault Pens	41	0.805	0.195	0.000	0.854	0.116	0.030	0.000	0.756	0.244	0.000	0.963	0.037	—	—
S. Tacoma Hatchery	84	0.845	0.155	0.000	0.947	0.047	0.006	0.000	0.625	0.375	0.000	0.800	0.200	0.988	0.012
Elwha Hatchery	50	0.880	0.120	0.000	0.940	0.050	0.010	0.000	0.656	0.344	0.000	0.810	0.190	0.989	0.011

[a]Complete set of allele frequencies for each sample is available in Reisenbichler and Phelps (1985).

TABLE 4. (Continued).

		Idh-3,4				*Ah-3*			*Pkg-2*			*Idh-2*		*bGa-1*	
Location	*N*	100	71	121	40	100	79	66	*100*	*114*	*150*	100	140	100	78
Wild fish															
Quillayute R.	494	0.628	0.260	0.023	0.089	0.918	0.080	0.001	0.382*	0.614	0.003	0.944	0.056	0.989	0.011
Hoh R.	302	0.678	0.182	0.026	0.114	0.891	0.096	0.013	0.374*	0.603	0.023	0.951*	0.049	0.987	0.013
Queets R.	559	0.658	0.212	0.020	0.110	0.888	0.104	0.008	0.415	0.579	0.006	0.967	0.033	0.997	0.003
Upper Quinault R.	66	0.648	0.187	0.031	0.134	0.924	0.076	0.000	0.549	0.441	0.010	0.962	0.038	0.970	0.030
N. Chehalis Basin	240	0.659	0.220	0.016	0.106	0.783	0.171	0.046	0.435	0.563	0.002	0.986	0.014	0.994	0.006
Skagit R.	61	0.691	0.236	0.023	0.050	0.796	0.194	0.009	0.463	0.451	0.085	—	—	0.992	0.008
Snow Cr.	84	0.672	0.188	0.045	0.096	0.893	0.095	0.012	0.511	0.477	0.011	0.970	0.030	1.000	0.000
Hatchery fish															
Soleduck (SR)	80	0.591	0.282	0.000	0.127	0.931	0.056	0.012	0.654*	0.346	0.000	0.981	0.019	1.000	0.000
Bogachiel Ponds	79	0.660	0.184	0.003	0.153	0.782	0.167	0.051	—	—	—	0.964	0.036	1.000	0.000
Quinault NFH	80	0.616	0.253	0.056	0.074	0.762	0.125	0.113	0.375	0.625	0.000	0.962	0.038	1.000	0.000
Quinault Pens	41	0.569	0.212	0.019	0.200	0.788	0.077	0.135	—	—	—	0.963	0.037	0.988	0.012
S. Tacoma Hatchery	84	0.736	0.156	0.033	0.075	0.866	0.091	0.043	0.368*	0.592	0.039	0.951	0.049	0.994	0.006
Elwha Hatchery	50	0.663	0.168	0.041	0.127	0.777	0.128	0.096	0.420	0.580	0.000	—	—	—	—

TABLE 5. Analysis of the variance among frequencies (q) of the common allele at each of seven loci or pairs of loci. Numbers are the values of the *F*-statistic with degrees of freedom in parentheses. Actual values used in the analysis were transformed frequencies ($\arcsin\sqrt{q}$). *$P < 0.05/n$, where n = number of loci, or pairs of loci, at which each contrast was tested.

Source of variation or contrast	Tests at individual loci						
	Ah-3	*Idh-3,4*	*Ldh-4*	*Mdh-3,4*	*MdhP-3*	*Pgk-2*	*Sod-1*
Variation within drainages	0.36 (9,5)	2.02 (8,5)	2.60 (9,5)	2.48 (9,5)	0.44 (9,5)	1.10 (7,4)	0.47 (9,5)
Variation among drainages	12.76 (4,4)	1.26 (4,4)	4.38 (4,4)	3.43 (4,4)	1.53 (4,4)	7.75 (4,1)	2.28 (4,4)
Hatchery versus wild							
Bogachiel Ponds	21.03 (1,5)*	0.07 (1,7)	9.17 (1,7)	11.50 (1,7)	9.72 (1,7)	—	0.40 (1,7)
Quinault NFH	28.75 (1,5)*	1.96 (1,7)	35.44 (1,7)*	1.72 (1,7)	21.83 (1,7)*	4.14 (1,1)	0.18 (1,7)
Quinault Pens	18.89 (1,5)*	5.83 (1,7)	11.81 (1,7)	0.01 (1,7)	15.54 (1,7)*	—	4.91 (1,7)
Soleduck	13.52 (1,5)	5.75 (1,7)	11.18 (1,7)	15.11 (1,7)*	11.53 (1,7)	62.24 (1,1)	4.45 (1,7)

TABLE 6. Gene diversity analysis (see Fig. 2) of steelhead from the Quillayute, Hoh, Queets, and Quinault rivers and northern Chehalis Basin based on 65 loci, of which 42 were monomorphic.

Source	Gene diversity	Relative gene diversity (%)
Average within samples	$H_s = 0.0518$	$H_s/H_t = 98.17$
Average between broods	$D_{bw} = 0.0002$	$G_{bw} = 0.31$
Average within major subdivisions of drainage	$D_{wm} = 0.0001$	$G_{wm} = 0.17$
Average among major subdivisions of drainages	$D_{md} = 0.0002$	$G_{md} = 0.34$
Average among drainages	$D_{dt} = 0.0005$	$G_{dt} = 1.02$
Total gene diversity	$H_t = 0.0528$	

Fish Hatchery (NFH) had an excess of heterozygotes at *Sod-1*. Both groups were juveniles taken from hatcheries, and these deviations probably reflected nonrandom mating in the hatcheries or small numbers of parents.

Variation between year-classes was significant for wild fish and for hatchery fish (Table 3). Seven loci, or pairs of loci, showed sufficient variation and were scored for enough fish to be used in the ANOVA (Table 4). Variation in allele frequencies within drainages was not significant (Table 5) so it was reasonable to pool samples within drainages, by year-class, for subsequent analysis with one-way ANOVA's. Variation in allele frequencies among drainages was not significant; however, hatchery fish were different from wild fish (Table 5).

Hierarchical analysis of genic diversity (heterozygosity) showed that the interbrood level accounted for 17% (0.31/(0.31 + 0.17 + 0.34 + 1.02; Table 6) of the genic diversity observed among samples; variation within major subdivisions of drainages accounted for 9%, variation among major subdivisions for 18%, and interdrainage variation for 55%. As with the ANOVA, the results indicated little genetic structuring within drainages (or possibly that our subdivisions of drainages did not match the actual genetic subdivisions); however, genetic differentiation between drainages was greater than that within drainages or between year-classes.

The UPGM analysis (Fig. 3) indicated three primary groups of fish: (1) summer-run fish from the Soleduck (hatchery) stock, summer-run fish caught at the mouth of the Quillayute River (hatchery fish from the Soleduck stock probably predominate in the catch at the mouth of the Quillayute River), and winter-run fish from Quinault Pens; (2) winter-run fish from Quinault NFH; and (3) fish from all remaining locations.

Discussion

The significant variation in allele frequencies among year-classes could have been partly the result of changes (caused by natural selection) in allele frequency within a year-class as fish grow older. Because we collected fish during a single year (1983), we could not test for the effect of selection separate from interbrood variation; however, Parkinson (1984) found no evidence of changes in allele frequency as fish grew older and we presumed that such changes did not occur in our fish. The significant variation among year-classes serves as a warning in genetic characterization studies. Clearly, fish should be taken from several year-classes, and statistical tests for differences between groups of fish should account for variation among year-classes. Collecting fish from throughout the population of interest (e.g. from throughout a drainage) should minimize the observed variation between year-classes, by ensuring representative samples, and thereby maximize statistical sensitivity.

We found no evidence for genetically distinct populations within drainages, and differences in allele frequencies between adjacent drainages along the north coast of Washington were

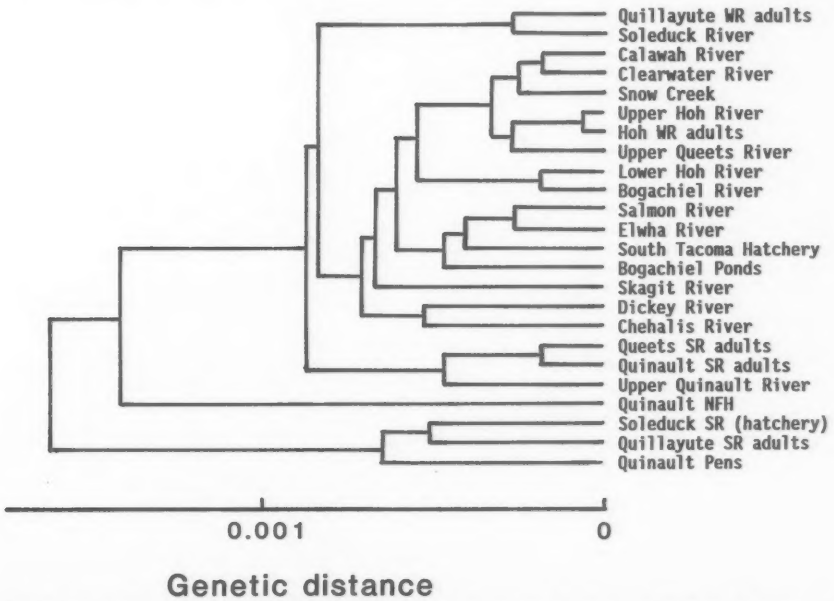

FIG. 3. Dendrogram showing results of analysis, by the unweighted pair group method, of genetic distance between samples. Distances (Nei 1972) were based on the 19 polymorphic loci or pairs of loci. (WR = winter-run; SR = summer-run).

not significant: the gene diversity analysis suggested genetic differentiation among drainages; however, tests for statistical significance are not available (Chakraborty and Leimar 1987). In contrast, Parkinson (1984) found highly significant differences among steelhead from adjacent streams throughout a large portion of British Columbia (he did not rigorously evaluate within drainage variation). Of course, statistical significance could have resulted from greater statistical sensitivity, so we modified our gene diversity analysis (by combining the major subdivisions level, the within major subdivisions level, and the brood level) to make our results directly comparable with those of Parkinson. Parkinson's values for genic diversity attributable to differences among adjacent drainages were subtantially greater than ours. His values (followed by ours in parentheses) were 0.024 for *Ldh-4* (0.001), 0.021 for *Sod-1* (0.003), 0.019 for *Mdh-3,4* (0.004), and 0.007 for *Idh-3,4* (0.001). A comparison of his results with ours suggests that (1) there is less gene flow among populations in British Columbia than along the north coast of Washington, (2) if these alleles are not selectively neutral, the environments are more similar among adjacent drainages in Washington than in British Columbia, or (3) gene flow is similar in Washington and British Columbia, but effective population sizes are substantially smaller and genetic drift correspondingly greater in British Columbia. The first hypothesis seems the most likely: gene flow from hatchery populations probably has had a much greater (homogenizing) effect in Washington than in British Columbia. Genetic variation among fish from the Washington streams may have been reduced because the hatchery fish have successfully spawned there. Allendorf and Phelps (1981), using electrophoresis to examine wild rainbow trout populations in Montana, found changes in allele frequencies caused by interbreeding with hatchery rainbow trout. When hatchery fish from a single population stray and successfully interbreed with wild fish from several wild populations, the genetic heterogeneity among the wild populations is reduced.

Others working with anadromous *Salmo* species have shown substantial variation in allele frequencies (Møller 1971; Ryman 1983; Parkinson 1984; Campton and Utter 1987) or quantitative genetic traits (Riddell et al. 1981; Refstie et al. 1977) among adjacent drainages or within drainages. Such results, along with the high degree of homing exhibited by anadromous *Salmo* species (e.g. steelhead; Shapovalov and Taft 1954), suggest that the basic breeding unit for these fish occurs at the intradrainage or interdrainage levels. The lack of statistically significant differences in our study does not preclude the existence of important genetic differences or status as separate stocks along the north coast of Washington. Smith (1968) has shown substantial genetic differences in several quantitative genetic traits between sympatric summer-run and winter-run steelhead, and Weber and Fortune (undated[2]) have shown genetic differences in season of return to freshwater between comparable summer-run and winter-run steelhead, despite the apparent lack of electrophoretically detectable differences (e.g. Chilcote et al. 1980). We recommend that steelhead from different drainages along the north coast of Washington be managed as distinct stocks.

The significant differences between hatchery fish and wild fish illustrate that few wild fish have been successfully included as brood fish in the hatcheries. The Quinault NFH population, in contrast with the other hatchery populations, was initiated with local fish, and we anticipated that it would be similar to the local wild fish. However, this population and the Quinault Pens population (derived from this population) were more distinct from wild fish than were the other hatchery populations (Fig. 3). Allendorf (1975) recorded frequencies of 0.856 for the common allele at *Mdh-3,4* and 0.575 at *Sod-1* for fish from the lower Quinault River (similar to our frequencies for Quinault NFH and wild fish from the north coast of Washington), but his frequency for the common allele at *Ldh-4* was 0.975, similar to our frequencies for wild fish but distinct from our frequency of 0.756 for Quinault NFH. We conclude that the differences between fish from Quinault NFH and wild fish resulted from the hatchery program. These changes could have been caused by the use of few spawners (male or female) in one or more years, artificial or natural selection associated with the hatchery, or interbreeding with fish transferred from other hatcheries or with strays from other remote stocks.

Artificial propagation is a potential threat to the conservation of anadromous salmonids because it can reduce the fitness of wild fish (Reisenbichler and McIntyre 1977; Chilcote et al. 1986) and reduce genetic differentiation among stocks (Allendorf and Phelps 1981; Reisenbichler 1984; Nelson and Soulé 1987). The predominant strategy for reducing this threat is to ensure that most hatchery fish return to a hatchery or collection facility (i.e. do not spawn in streams with wild fish) and that hatchery fish are genetically similar to wild fish (see e.g. Helle 1981). Our results show that a hatchery stock developed from a wild stock can diverge substantially from the wild stock if wild fish are not continually included in the hatchery brood stock.

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Effect of Carbon Monoxide Exposure on Gas Bubble Disease in Rainbow Trout (*Salmo gairdneri*)

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Cumulative mortalities of 100, 50, 20, and 0% due to gas bubble disease (GBD) occurred on exposure of rainbow trout (*Salmo gairdneri*) for 180 min to supersaturated water at 136, 130, 124, and 116% total dissolved gas saturation (TDGS), respectively. At 130% TDGS, a prior exposure to carbon monoxide (CO), which converted 80% of the hemoglobin to carboxyhemoglobin (COHb), significantly prolonged survival time, but cumulative mortality was insignificantly reduced in the same group. Histologically, all supersaturation mortalities had branchial lesions for GBD (characterized by gas displacement of blood from the afferent arterioles of the gill filaments), while 70% had gas emboli in the retinal choroid gland. At a TDGS of 100%, these histological lesions were not observed and the CO 80% COHb conversion did not induce mortality within 120 min. Breathing movements of both control and CO-exposed fish slowed greatly when a TDGS of 130% was imposed; however, the COHb conversion initiated tachypnea and the CO-treated fish maintained a relatively higher respiratory rate when exposed to supersaturation conditions for a period which was proportional to their prolonged survival time. We concluded that the initial formation of gas emboli preceding fatal GBD was delayed as a result of a CO-inhibited function of hemoglobin.

Des taux cumulatifs de mortalité respectifs de 100 %, 50 %, 20 % et 0 % attribuables à l'embolie gazeuse ont été observés chez la truite (*Salmo gairdneri*) après une exposition de 180 min à une eau sursaturée à raison de 136, 130, 124, et 116 % de gaz totaux dissous. À une saturation de 130 %, une exposition antérieure au monoxyde de carbone (CO), qui a converti 80 % de l'hémoglobine en carboxyhémoglobine (COHb), a prolongé considérablement le temps de survie, mais n'a réduit la mortalité cumulative que de façon insignifiante dans le même groupe. Du point de vue histologique, tous les poissons morts par sursaturation présentaient des lésions branchiales de l'embolie gazeuse (caractérisées par un déplacement gazeux du sang des artérioles afférentes des filaments des branchies) tandis que 70 % présentaient une embolie gazeuse dans la glande choroïdienne de la rétine. À une sursaturation de 100 %, ces lésions histologiques n'ont pas été observées et la conversion de 80 % du CO en COHb n'a pas entraîné de mortalité à une exposition inférieure à 120 min. Les mouvements respiratoires des poissons témoins et des poissons exposés à CO étaient très évidents dans l'eau sursaturée à 130 %; toutefois, la conversion en COHb déclenchait une tachypnée et les poissons traités au CO affichaient un rythme respiratoire relativement plus élevé lorsqu'ils étaient exposés à des eaux sursaturées pendant une période proportionnelle à leur temps de survie prolongé. Nous en avons conclu que la formation initiale de l'embolie gazeuse précédant le traumatisme gazeux fatal était retardée par suite d'une inhibition de l'hémoglobine par le CO.

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Gas bubble disease (GBD), also called gas bubble trauma, in hatcheries is typically associated with water from wells which have had air introduced by the pumping process or from springs that have naturally become supersaturated with gases. In a natural body of water, changes in water flow, temperature, or biological homeostasis may result in total dissolved gas saturation (TDGS) of over 100%, a supersaturation condition that may result in GBD. Commonly, in spring runoff flooding, cascading river water plunges over dams deep into the post-dam pool and the pressure change results in elevation of TDGS.

Fish with GBD, both in hatcheries and in the natural watershed, have a similar pathologic presentation. The formation and enlargement of microbubbles in the blood of fish exposed to supersaturated water is responsible for gas emboli

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which block the circulation and cause death. GBD may occur whenever air causes total dissolved gases to exceed a critical level of saturation; however, O_2 alone may supersaturate water without causing GBD mortality to exposed salmonid fish. If relatively low TDGS exists in supersaturation (viz. 111–116%), both O_2 and N_2 supersaturation are necessary to induce GBD, and supersaturation of one or the other is not fatal (Machado et al. 1987).

In previous studies (Bell et al. 1986; Machado et al. 1987), it was demonstrated that lesions are not randomly distributed but always have a characteristic pattern associated with specific tissues in rainbow trout (*Salmo gairdneri*) affected with GBD. This pattern appeared to be related to the O_2 requirements of tissues, and the gills, brain, retina, and base of fins were invariably the earliest site of embolism or emphysema formation. Histologic examination of the development of small gas emboli in the retinal choriocapillaries revealed the progressive development of exophthalmia or "pop eye" (Machado et al. 1987). Other workers have shown that salmonid Root effect hemoglobins (REH) are involved in the normal O_2 secretion mechanism of the choroid gland of the eye (Ingermann 1982) and that oxygenated REH can unload O_2 against high O_2 tensions when blood is exposed to the low pH of the choroid gland.

The present study was designed to test the hypothesis (Bell and Farrell 1972) that an accelerated O_2 unloading from oxyhemoglobin during exposure to supersaturation conditions initiates GBD. To accomplish this, fish pretreated with carbon monoxide (CO) were compared with control fish during a standarized supersaturation exposure. CO combines with trout ferrohemoglobin and interferes with O_2 transport by two major mechanisms. One mechanism is by displacement of O_2 from hemoglobin in the arterial blood, thereby decreasing the O_2-carrying capacity of the hemoglobin. The other is by interference with O_2 release in the tissues through alteration of the shape of the O_2 hemoglobin dissociation curve. Holeton (1971a) calculated that in trout the affinity of CO for hemoglobin is about 66 times greater than of O_2, but the specific affinity for REH has not been precisely determined (Brunori 1975). Even though trout are more sensitive to the loss of functional hemoglobin than some other aquatic species, they are able to survive by utilizing O_2 transported through the plasma (Holeton and Randall 1967b; Stevens and Randall 1967b).

In the present study, mortality, lesion development, and respiratory rate were recorded. It was anticipated that a CO-induced hemoglobin dysfunction could prevent the development of GBD.

Materials and Methods

Experimental Animals

Rainbow trout fry were obtained from the Baldwin Fish Farm, Baldwin, Michigan, and were transported to Michigan State University. Fish were fed twice a day to satiety with Purina Trout Chow and exposed to artificial light which simulated a natural photoperiod (14 h light and 10 h dark).

One thousand rainbow trout (8 wk old, average total length of 4.0 cm) were maintained in a holding tank provided with well water. The fish were acclimated in the holding tank for 2 wk before the beginning of the experiments.

The well water had the following chemical and physical characteristics: hardness (as $CaCO_3$), 310 mg/L; alkalinity (as $CaCO_3$), 306 mg/L; pH 7.5 – 7.7; and temperature, 12°C.

Experimental Series

A pilot study and a series of experiments, I–IV, were conducted. The pilot study superficially examined the effect of CO treatment on survival and enabled approximate mortality calculations in fish treated with CO and exposed to supersaturation at a TDGS of 133% for 90 min utilizing a similar experimental design and the same apparatus as described below. The results revealed an apparent protection from mortality by CO treatment. The remaining experiments were designed to establish the nature of the effect.

To determine the precise time necessary to supersaturate a specific volume of water so that trials would involve an appropriate exposure, supersaturation was produced by pressurizing a 25-L plastic capped jar filled with 10 L of well water at 12°C with compressed air (20.68×10^4 N/m^2). The jar was constantly agitated by an electric shaker at 110 rpm. The water was then depressurized and transferred to an open tank creating a supersaturation. Pressurization times of 5, 10, 15, and 20 min were used. These experiments revealed that it took between 5 and 10 min to air-supersaturate the water. An additional period of pressurization did not result in any increase in supersaturation. Thus to insure a consistent level of supersaturation the pressurization time was 15 min. The volume of 10 L of water was utilized because tests previously had shown that 10 trout fry can survive for at least 48 h in 10 L of static, normally saturated water.

An electronic Tensionometer (model 300C, Novatech Designs Ltd, Victoria, British Columbia, Canada) was used to measure the total gas pressure (TP, in millimetres of mercury) of all dissolved gases in the water and the ambient barometric pressure (B). The TDGS in percentage was calculated according to the following equation:

$$\text{TDGS (\%)} = \frac{100\,(\text{TP} + B)}{B}.$$

Series I experiments

To maximize the probability of detecting an effect of CO exposure, we measured the TDGS level that killed about 50% of control fish. Four tests were conducted at various supersaturation levels achieved by pressurization as described above for 15 min at 25.3×10^4, 15.1×10^4, 12.6×10^4, and 5.0×10^4 N/m^2. For each of these tests, 10 fish were placed in 10 L of supersaturated water at 12°C, and the mortality was recorded every 5 min during 180 min of observation.

Series II experiments

We determined the carboxyhemoglobin (COHb) conversion of fish exposed to an air mixture containing 3% CO (certified standard, Union Carbide Co.) for varying periods. The efficiency of conversion was determined by measuring the saturation of COHb in blood after exposure. Because it was technically impossible to take enough blood from 8-wk-old fish for this purpose, adult rainbow trout were used so that 1 mL of blood could be drawn. Before placing the fish in the tank, 10 L of water at 12°C was saturated with the gas mixture through an aquarium air-diffusing stone for 15 min at a flow of 80 mL/min. Thereafter, fish were placed in the tank, and blood samples for each of the five tests were taken after 15, 30, 60, 90, and 120 min of CO exposure. Immediately after removing the fish from the tank, samples of blood were taken by cardiac puncture using 2.5-mL-capacity blood gas syringes containing 125 USP units of lyophilized lithium heparin (Terumo Co.,

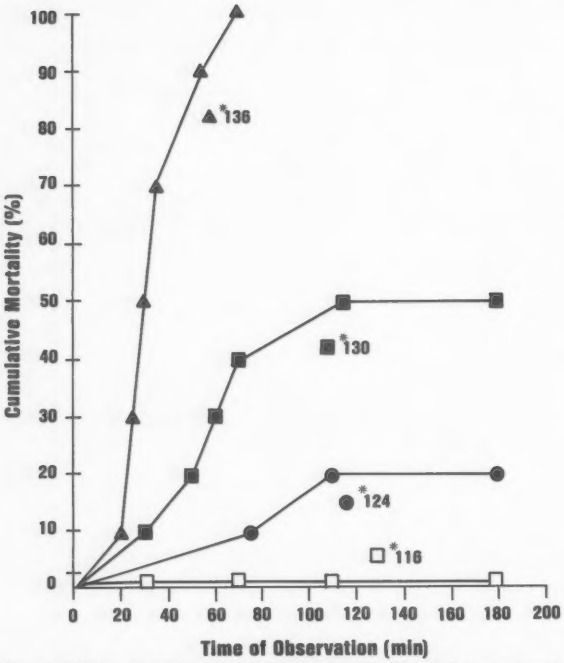

FIG. 1. Effects of various levels of supersaturation (asterisks) expressed as TDGS by air on the mortality of rainbow trout fry (n = 10) exposed to 10 L of water at 12°C during 180 min.

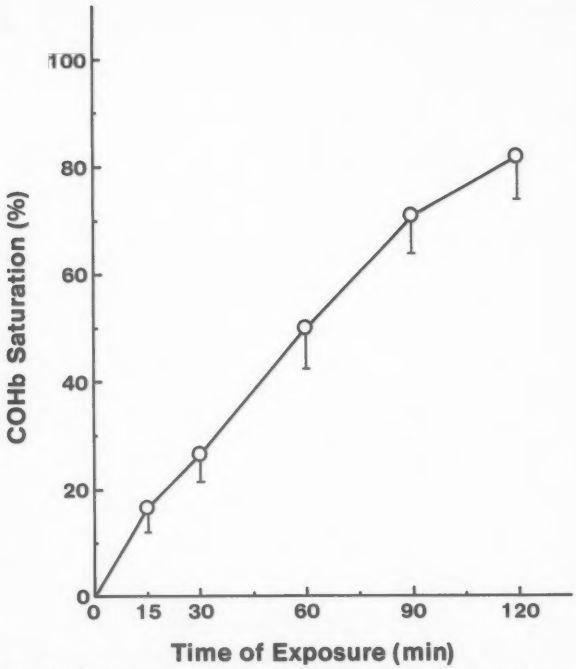

FIG. 2. Concentrations (± SD) of COHb of adult rainbow trout (n = 5) venous blood after 15, 30, 60, 90, and 120 min of exposure to 10 L of water at 12°C containing 3% CO diffused into the water through an air stone at a flow rate of 80 mL/min. Line points indicate percent COHb saturation.

Tokyo, Japan) and 18-gauge, 38-mm hypodermic needles. Fish were immobilized in a wet towel, and care was taken to assure that the time that elapsed until blood collection was less than 1 min. Samples were immediately placed on ice and were transported within 3 h to the clinical chemistry laboratory at St. Lawrence Hospital, Lansing, Michigan, for blood gas analysis. The percent saturation of COHb in deoxygenated whole blood samples was determined by the method of Siggaard-Andersen (1977) using the OSM-2 Hemoximeter (Radiometer America Inc., Westlake, Ohio).

Series III experiments

We examined the effects of blocking oxyhemoglobin formation by CO exposure to determine if the hemoglobin function influenced GBD. In this phase of the investigation, both the control and CO treatment groups of fish were exposed to supersaturated water. First, the CO exposure and the sham exposure for controls were conducted concurrently for 120 min under identical conditions. The CO treatment protocol was the same for these experiments as it was for Series II, including the water volume, temperature, and the flow for CO injection. Twenty rainbow trout were used in each of 10 trials: 10 CO exposed and 10 controls.

For each trial, randomly selected fish were CO treated and then treatment fish were simultaneously tested with control fish to eliminate minor inconsistencies between the groups. Because stress may precipitate GBD, the sides of the tanks were covered with sheets of black polyethylene, and particular care was taken to avoid any sudden movements.

In the CO treatment groups, after saturating the water with an air mixture containing 3% CO, 10 fish were placed into the tank, and the same mixture was added at a fixed rate of 80 mL/min for 120 min. In the control groups, after saturating the water with air, 10 fish were placed into the tank and air was added at a rate of 80 mL/min for 120 min. Then, both treatment and control fish were transferred to other identical tanks containing 10 L of supersaturated water at 130% TDGS. The duration of each supersaturation test was 180 min.

Fish were observed every 5 min throughout each 180-min trial for mortality and the dead fish were removed from the tank. Five moribund fish from each of the four groups were fixed by the technique of Bell et al. (1986) and then sectioned and stained with hematoxylin and eosin for histopathological examination.

The tests were performed under static flow conditions, and the total gas pressure could be measured only at the end of each trial because the use of the tensionometer requires a frequent shaking of the instrument for proper readings. If measurements had been taken continually during the tests, the manual agitation of the water would have inconsistently reduced the supersaturation level. It had previously been determined that the exposure of the supersaturated water to atmospheric pressure for 180 min would decrease the TDGS by 5%.

Series IV experiments

Because of observations of altered respiration in previous tests, we examined the respiratory performance of trout using the same CO and supersaturation exposure and protocol described for Series III experiments (TDGS = 130%). The breathing rates (buccal and opercular) of four fish groups were recorded with the aid of a stopwatch and the measurement was the average of three or more determinations. Significant disturbance to the fish was avoided by simply placing a black poly-

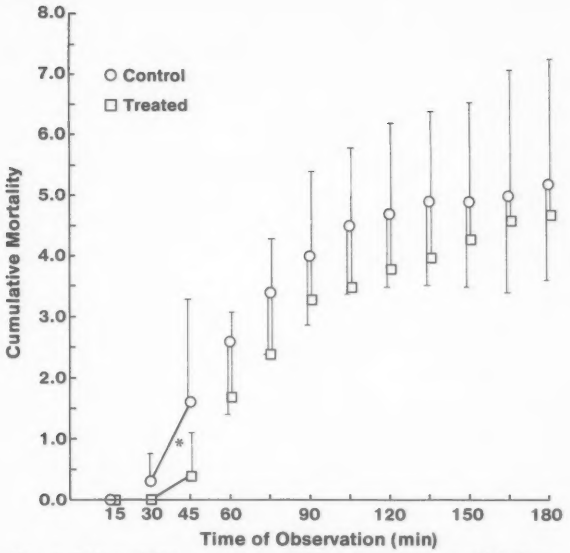

FIG. 3. Effect of CO treatment on GBD susceptibility at 12°C (Series III experiments). Treated fish were exposed to water containing 3% CO for 120 min prior to supersaturation. CO-treated fish and controls were placed in water supersaturated to 130% TDGS. The mortality data represent 10 trials of 10 fish each and the lines were plotted by computing the cumulative mortality for CO-treated and control groups at 15-min intervals during 180-min observations. The slopes of the lines are significantly different (asterisk).

ethylene sheet with peep-hole alongside the tanks. Lighting was supplied by overhead fluorescent lamps placed 0.25 m from the water surface. After observing the control and CO-treated test subjects in normally saturated water for 120 min, supersaturation was imposed.

Statistical Analysis

Statistical analysis for Series III experiments was performed utilizing a two-way factorial analysis of variance (SAS) with postanalysis comparison of means utilizing the least significant difference (LSD) test, at $P < 0.05$ for significance.

Analysis of mortality data was performed by comparing control and treated groups. The mortality data obtained during the 180-min observation from the 10 trials were pooled and divided into 12 blocks of 15 min each. For each of these intervals, variable difference between treated versus control mortality was assessed by means of Student's *t*-test ($P < 0.05$).

Results

Series I

Because of the pilot study results, it appeared that the most sensitive detection of a CO treatment effect would be obtained by having a cumulative mortality of about 50%. For this purpose, Series I experiments were conducted to examine the mortality rate for fish under the conditions to be utilized for CO treatment tests. By varying the level of supersaturation with different injection pressures, the mortality data for 10 fish groups exposed for 180 min in 10 L of water (Fig. 1) demonstrate that the lowest TDGS level for 100% mortality was 136% (25.3×10^4 N/m^2), for 50% mortality was 130% (15.1×10^4 N/m^2), for 20% mortality was 124% (12.6×10^4 N/m^2), and for the highest level that killed no fish was 116% (5.0×10^4 N/m^2).

Series II

Because there was no available information about the rate of COHb conversion under the conditions of these tests, the Series II experiments determined the percent saturation of COHb of adult trout blood exposed to 10 L of water equilibrated with an air mixture containing 3% CO (Fig. 2). The data show that COHb conversion increased progressively with exposure time, reaching 82% after 120 min. Fish exposed for a longer period evidenced narcosis with loss of equilibrium. Because of the blood sample size requirement of 1 mL, COHb conversion was determined in large trout. Thus, an extrapolation was necessary to arrive at the conclusion that approximately 80% of the hemoglobin in small fry similarly exposed would be converted to COHb.

Series III

Mortality data (Fig. 3) comparisons for each of the 15-min intervals indicated significant ($P < 0.01$) difference between CO-treated and control groups for the 30- to 45-min interval of exposure. However, the analysis of variance indicated that the accumulated total mortality of the treatment group was not significantly different from controls at $P < 0.05$.

Branchial lesions confirmed the cause of death to be GBD and were characterized microscopically by a gas displacement of blood in the arterioles of the gill filaments. Gas emboli were evident in 80 and 60% of the retinal choroid gland sections of the control and CO-treated fish, respectively, that succumbed to supersaturation treatment (Fig. 4). Similar extent and distribution of lesions were found in CO-treated and control moribund fish with GBD, but no lesions of gas embolization were detected in any CO-treated or control fish prior to supersaturation exposure.

Series IV

The respiratory rate of control fish declined from 104 to 90/min during the initial 120-min observation and dropped to about 20/min immediately after being transferred to supersaturated water. Breathing movements remained slow for about 30 min until the onset of convulsions. By comparison, CO-treated fish had a higher initial respiratory rate of 120/min with a gradual decrease to 82/min after the 120-min rest period. When CO-treated fish were transferred to supersaturated water, the respiratory rate declined but was twice the control fish rate for a period of 15–30 min (40 versus 20/min).

Discussion

In defined conditions of a trout-rearing facility, the air supersaturation of water at 111–115% TDGS constitutes an approximate GBD lethal dose (LD_{50}) in 20 d for 3-mo-old trout (Machado et al. 1987). In the present experiments, fish of the same size were exposed in the laboratory to 130% TDGS in order to determine the role of hemoglobin function in the pathogenesis of GBD. The conditions that were imposed resulted in 50% mortality within 2 h. Dysfunctional hemoglobin was produced in one group by CO treatment before supersaturation exposure, and this resulted in 80% circulating COHb. As a

FIG. 4. (A) Normal rainbow trout eye and (B) GBD rainbow trout eye with a
Banded pattern in both specimens is the retina. H&E stain. Bars = 20 μm

e with a gas embolism within the choroid rete mirabile venous drainage (asterisk).
20 μm.

result of CO treatment, the onset of mortality was delayed for approximately 25 min and the completion of mortality was delayed by approximately 30 min (165 versus 135 min). These observations are corroborated by a positive test for significant difference in mortality between the groups ($P < 0.01$) at the 30- to 45-min time interval as demonstrated in Fig. 3 by the difference in the slopes of the time interval lines. After the delay, formation of intravascular bubbles in CO-treated fish did occur, and once initiated, neither the rate nor morbidity of GBD differed significantly from that of controls as demonstrated in Fig. 3 by the similar slopes of time interval lines.

A novel observation which may be important in the pathogenesis of GBD is that, prior to the onset of GBD, markedly reduced ventilation occurred in control fish exposed to supersaturation; a similar but blunted bradypnea occurred in CO-treated fish. It has been reported previously that CO exposure greatly increases breathing movements and cardiac output in rainbow trout (Holeton 1971b), and it appeared that this may have been responsible for the more rapid ventilation rate in CO-treated fish.

In the present experiments, the control group respiratory rate declined to 22% of the preexposure rate (90/min) in supersaturation conditions. Breathing movements remained slow for 25 min until the onset of fatal GBD. However, CO-treated fish maintained their respiratory rate at 40% of their preexposure rate, a rate twice that of exposed controls.

Trout have been shown to increase their ventilatory rate during CO treatment because of the need to maintain tissue O_2 delivery in the face of the decreased O_2-carrying capacity of COHb (Holeton 1977). The rapid respiration is also associated with hypertension (Holeton and Randall 1967a; Stevens and Randall 1967a). During these compensating respiratory and circulatory processes, the CO_2 is excreted through the gills, helping to maintain the blood pH level within the physiological range. Blood pH remains constant in spite of the CO-induced hypoxia (Holeton 1977). For this reason, we have been encouraged to further examine the hypothesis that it is a transiently higher ventilation rate in the CO-treated fish that may enable longer survival during supersaturation exposure.

The supersaturation-induced stress in fish may be associated with acidemia. The association of altered blood and red blood cell (RBC) pH with the onset of the GBD was examined during supersaturation exposure (Machado et al. 1988). The investigation of selected hematological parameters revealed that GBD was preceded by a RBC pH drop below 7.00 (approximately 6.90).

We conclude from this study and other accumulated evidence that the initiation of fatal embolus formation is significantly influenced by oxyhemoglobin function, since an 80% COHb formation prolonged the survival time. That there was no significant change in the accumulated mortality may have been because a conversion of 80% is just not enough to prevent the eventual formation of lethal emboli by the remaining 20% unconverted oxyhemoglobins. One might infer from these findings that CO-exposed fish tested at a minimal, lethal exposure for GBD would survive indefinitely.

The physical–chemical explanation of how gas bubbles are formed within solutions remains incompletely resolved. The assumption that bubbles propagate from preexisting gas nuclei is well accepted (McLeod 1978), but how the initial nuclei are generated in various systems is still unknown. In microbial models, intracellular gas bubble formation apparently requires the presence of gas nuclei a priori or decompression from pressures above 152×10^5 N/m^2 for internal bubble nucleation to occur (Hemmingsen 1982). GBD in fish occurs at relatively low TDGS, much less than a 2.03×10^5 N/m^2 differential pressure. In fish, therefore, it should come as no surprise that a search is on for an intrinsic mechanism which might explain the generation of blood gas nuclei in GBD.

The results of this and our preliminary studies (Bell et al. 1986; Machado et al. 1987, 1988) on the pathogenesis of gas embolism support the hypothesis that rapid release of O_2 from hemoglobins may be the mechanism responsible for gas nuclei generation.

The eye of many teleosts, including trout, requires O_2 tensions approximately 20 times higher than those of blood or ambient water, and therefore, a specialized secretory mechanism has evolved (Wittenberg and Wittenberg 1974). As evidenced by earlier studies (Machado et al. 1987) and by lesions in 70% of GBD fish in this study (Fig. 4), the result of the ocular secretory mechanism is the deposition of intravascular gas within the choroid gland during supersaturation exposure. In trout, oxygenated REH can unload O_2, against high O_2 tensions, when exposed to low pH (Ingermann 1982). It may be this process in the choroid rete of the eye, which normally supplies high levels of O_2 to the retina, that is responsible for the localized lesion in the eye of fish affected with GBD.

This mechanism involves secretion of lactic acid through aerobic glycolysis by the cells of the retina and release of lactic acid into the choroid rete (Fairbanks et al. 1974). The lactic acid production lowers the pH of entering blood, forcing oxyhemoglobin to rapidly unload O_2 which then diffuses from the choroid rete into the retina. It is inferred that embolization may occur at this point in GBD. Due to the anatomical configuration of the choroid rete, a countercurrent effect causes rediffusion of lactic acid and O_2. A high concentration of O_2 in the choroid rete capillaries is maintained by the Root effect and salting out (Fange 1983). Normally, O_2 and lactic acid diffuse from the efferent into the afferent capillaries, maintaining O_2 secretion to the retina.

The significance of understanding the mechanism of initial gas nucleation is that, if the REH are responsible for the initiation of GBD in conditions that are physiologically important, then the resistance reported for certain fish (Cramer and McIntyre 1975) may be explained. If specific adaptive responses in hemoglobin function confer resistance, then the resistant stock could be identified in the field at the time of selection of broodstock.

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Influence of Environmental Factors and Piscivory in Structuring Fish Assemblages of Small Alberta Lakes

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Robinson, C. L. K., and W. M. Tonn. 1989. Influence of environmental factors and piscivory in structuring fish assemblages of small Alberta lakes. Can. J. Fish. Aquat. Sci. 46: 81–89.

We surveyed 45 small lakes in central Alberta to determine if discrete, repeatable types of fish assemblages exist, to identify the main environmental and biotic processes likely responsible for assemblage-level patterns, and to compare and contrast Alberta patterns with those observed in other regions of North America. Overall, 11 species of fish were caught in 36 lakes; nine lakes were fishless. Hierarchical classification and detrended correspondence analysis of fish species presence/absence identified two main assemblage types, characterized by northern pike (*Esox lucius*) and yellow perch (*Perca flavescens*) versus brook stickleback (*Culaea inconstans*) and fathead minnow (*Pimephales promelas*). Pike/perch lakes were significantly deeper and larger than lakes of the stickleback/fathead assemblage type; however, a subset of the former group lacking yellow perch was environmentally similar to stickleback/fathead lakes. Piscivory by northern pike appears to be the dominant process maintaining nearly complete negative associations between members of the two assemblage types. Despite environmental and faunal-richness differences, our results were not unlike those from southern Ontario and northern Wisconsin. For small boreal lakes of North America, piscivory and processes related to a small number of environmental variables, such as maximum depth, surface area, and isolation, appear to be most important in structuring fish assemblages.

Nous avons étudié 45 petits lacs du centre de l'Alberta pour déterminer s'il y existait des types de groupements de poissons discrets et répétables. Les objectifs étaient d'identifier les principaux processus écologiques et biotiques susceptibles d'être à l'origine des divers types de groupement et de comparer les tendances observées en Alberta avec celles d'autres régions de l'Amérique du Nord. Au total, onze espèces de poisson ont été capturées dans 36 lacs; neuf lacs ne comptaient aucun poisson. La classification hiérarchique et l'analyse des correspondances faisant abstraction des tendances de la présence/absence des espèces de poisson ont permis d'identifier deux principaux types de groupement, l'un caractérisé par le grand brochet (*Esox lucius*) et la perchaude (*Perca flavescens*) et l'autre, par l'épinoche à cinq épines (*Culaea inconstans*) et la tête-de-boule (*Pimephales promelas*). Les lacs fréquentés par le groupement brochet/perchaude étaient beaucoup plus profonds et plus étendus que les lacs abritant le groupement épinoche/tête-de-boule; toutefois, nous avons constaté qu'un sous-groupe de lacs renfermant le premier groupement mais où la perchaude était absente était similaire sur le plan écologique aux lacs fréquentés par le groupement épinoche/tête-de-boule. Il semble que ce soit principalement en raison du fait que le grand brochet soit un poisson ichtyophage que les membres de ces deux types de groupement ne vivent presque jamais en association. En dépit des différences constatées tant sur le plan écologique que sur celui de la richesse faunique, nos résultats ne diffèrent guère de ceux obtenus dans les lacs du sud de l'Ontario et du nord du Wisconsin. Dans les petits lacs de la zone boréale de l'Amérique du Nord, l'ichtyophagie et les processus liés à un petit nombre de variables environnementales, comme la profondeur maximale, la superficie et l'isolement des lacs, semblent constituer les facteurs qui revêtent le plus d'importance dans l'étude des structures des groupements de poissons.

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Glaciated regions of North America contain a large number of small lakes that are potentially available for fish to inhabit. Results of community studies from north-central districts of the continent suggest that these lakes do not contain random mixtures of the regional fish fauna but rather a small number of assemblage types (e.g. Harvey 1978, 1981; Tonn and Magnuson 1982; Rahel 1984; Eadie and Keast 1984). With the exception of Eadie and Keast (1984), these studies have examined only single regions and have generally focussed on local factors, such as autecological responses to the abiotic environment; as a result, they have tended to ignore regional–historical events (Henderson 1984; see also Ricklefs 1987).

Abiotic conditions can limit the composition of fish assemblages to species tolerant of the physico-chemical environment of small lakes. Factors such as low pH (Harvey 1975; Rahel and Magnuson 1983; Rask 1984a, 1984b; Rahel 1986; Rago and Weiner 1986) and low winter oxygen concentrations (Tonn and Magnuson 1982) can be directly linked to the absence of local populations because of physiological intolerance. In other cases, behavioral responses to deteriorating environmental conditions allow some species to temporarily or permanently maintain populations in lakes from which they would otherwise be excluded (Tonn and Magnuson 1982; Magnuson et al. 1985).

Other environmental factors, such as those associated with habitat heterogeneity (Eadie and Keast 1984), productivity (Gascon and Leggett 1977), and lake size (Harvey 1978; 1981; Eadie et al. 1986), are believed to influence fish assemblage composition indirectly via their effects on biotic interactions (competition and predation). Factors related to dispersal also influence the structure of fish assemblages in small lakes (Tonn and Magnuson 1982); lakes more isolated from a stable source of species (e.g. a large lake or river) are predicted to have lower

species diversity than lakes closer to the source. Assemblage composition may also differ due to differential dispersal abilities among species (MacArthur and Wilson 1967; Magnuson 1976).

Although abiotic conditions clearly limit fish species distributions and occurrence, species are often absent from seemingly suitable and accessible habitats. Competition and predation may be of equal or greater importance than abiotic factors in structuring assemblages. In some small lakes of Ontario and Wisconsin, for example, the paucity of cyprinids and darters is thought to be a result of predation and competition from centrachids, northern pike (*Esox lucius*), and yellow perch (*Perca flavescens*) (Harvey 1981; Tonn and Magnuson 1982; Rahel 1984).

The occurrence of repeatable local fish assemblages and their correlation with abiotic and biotic conditions of the lakes in which they occur provide an empirical vehicle for understanding and predicting how internal and external elements (e.g. eutrophication, species additions) influence and structure fish assemblages (Kerr 1982; Rigler 1982). However, studies that have successfully identified patterns in fish assemblage structure and related them to abiotic and biotic structuring mechanisms have been primarily restricted to regions that are relatively similar (e.g. southern Ontario and northern Wisconsin), both environmentally and in terms of the richness and composition of their fish faunas. Can similar assemblage-level patterns be found and do similar assemblage–environment relationships exist in less species rich and environmentally different regions of North America?

With 35 species (Paetz and Nelson 1970), Alberta's Athabasca River drainage basin has a relatively low total diversity of fishes when compared with many areas of north-central North America (e.g. >75 species in northern Wisconsin; Becker 1983). This low species diversity is thought to be due primarily to long distances from major glacial refugia (Lindsey and McPhail 1986), as well as climatic limitations (e.g. Shuter et al. 1980). The majority of the central Athabasca drainage flows through woodland plains over luvisolic, alkaline soils underlain by upper Cretaceous sandstone, shales, and coal (Hardy 1967). As a result, most small lakes within this region differ from areas of north-central North America with respect to many limnological characteristics, such as nutrients, color, and acidity (Riley and Prepas 1984; Chambers and Prepas 1988). As well, many lakes in central Alberta have only intermittent inlet/outlet streams as a result of low topographic relief and high evaporation and low precipitation rates (Fisheries and Environment Canada 1978; Chambers and Prepas 1988). It seems unlikely that some environmental factors identified as important in north-central North America (e.g. low pH) are equally important in structuring assemblages in small Alberta lakes, whereas other factors (e.g. surface area and isolation) may have similar effects on Alberta assemblages because of a seemingly ubiquitous affect on vertebrate species composition (e.g. MacArthur and Wilson 1967).

To evaluate the general importance of factors previously identified as influencing fish assemblages in small north-temperate lakes, we determined species composition and richness and examined their relationships with environmental conditions for small lakes of the Athabasca River drainage. Specifically, we addressed the following questions: (1) Are there discrete, repeatable assemblages of fish in these small lakes? (2) To what extent is the pattern of fish assemblage structure related to abiotic factors of the lakes' environment? Can these be linked with specific deterministic structuring mechanisms? (3) To what extent does piscivory contribute to the structure of the assemblages? (4) How do structuring mechanisms for fish assemblages in small lakes of central Alberta compare with those identified for lakes of north-central North America?

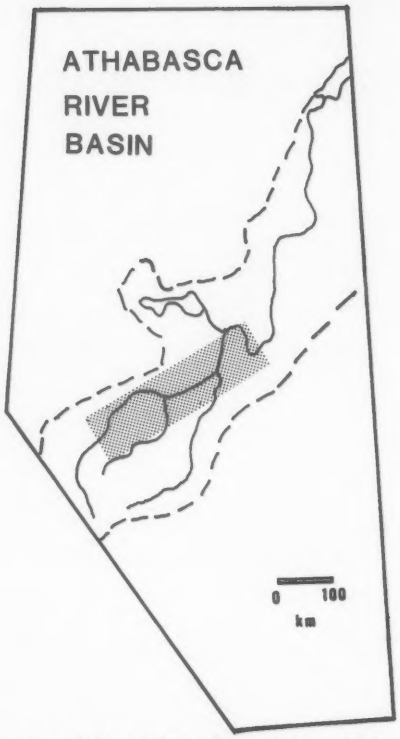

FIG. 1. Location of the Athabasca River drainage basin in central Alberta. The 45 study lakes are located within the shaded area.

Methods and Materials

Lake Selection and Sampling

Forty-five lakes <100 ha were selected from accessible areas of the south-central portion of the Athabasca River basin (Fig. 1). From May through August 1986 and 1987, we determined fish species composition in each lake by using a combination of gear set overnight. Forty unbaited minnow traps (44.5 cm long, 23 cm in diameter) and five fyke nets (9.1 × 0.9 m lead, 6.0-mm mesh, and 76-cm-diameter hoops) were set overnight in the littoral zone. Two experimental gill nets (38 × 1.8 m, with 13-, 19-, 25-, 31-, and 38-mm mesh) and a trammel net (37 × 1.2 m, with 20-cm walls and 25-mm mesh) were set parallel to shore along the 2-m contour. Six 15-min sweeps of a beach seine (15.2 × 1.8 m, 5-mm mesh) were performed on hard bottomed areas. Fishes caught with the above gear were identified to species (Paetz and Nelson 1970), counted, and returned to the lake. A sample collection of each minnow species was retained for verification of identification. Similar protocol has been shown to be effective in sampling fish assemblages in small lakes of other regions (Harvey 1981; Tonn and Magnuson 1982; Rahel 1984). However, to measure the repeatability of fish sampling for this set of lakes, a subset

of six lakes was resampled, at various levels of intensity with the above gear, during May and August of 1986 and 1987.

For each lake, conductivity and pH were measured in situ from vertical profiles of at least six midwater stations using a Hydrolab Surveyor II Water Quality Monitor. Depth and Secchi depth were also determined at each station and depth at the deepest station was used as maximum depth. Water samples, collected from 0.5 m at the center of a lake, were analyzed for apparent water color and total dissolved phosphorus (E. E. Prepas, Department of Zoology, University of Alberta, Edmonton, Alta., unpubl. guide). Water samples and in situ measurements were collected once for each lake between 1100 and 1300 during July or August. For a subset of 10 lakes we were able to compare our limnological measurements with results of an independent survey (Prepas et al. 1988). Surface area, shoreline length, and elevation were obtained from 1:50 000 topographical maps.

Macrophyte diversity and relative abundance were estimated visually and from grab samples at three stations along each of six randomly placed transects running perpendicular to shore. The relative isolation of a lake was determined by the absence of an inlet/outlet (score: 0), presence of an intermittent inlet/outlet (score: 0.5), or presence of a permanent inlet/outlet (score: number of outlets $\times$ 2). We considered a permanent inlet/outlet to be much more important than an intermittant connection because of increased potential for changes in assemblage structure due to immigration and emigration of fish species. Outlet information for each lake was obtained from 1:50 000 topographic maps (1974–76) and 1:100 000 county maps (1985–86) and verified in the field.

Data Analysis

A fish species was included in a lake's assemblage if we caught >10 individuals of at least two size-classes (e.g. juveniles and adults). To analyze for patterns of fish assemblage composition among lakes, we subjected the lakes-by-species (presence/absence) data matrix to two methods of multivariate community analysis: hierarchical classification using agglomerative, unweighted group averaging and Jaccard's coefficient of similarity (Gauch 1982; Pielou 1984) and detrended correspondence analysis (DCA; Hill and Gauch 1980).

To investigate how individual species responded to specific lake features, we analyzed distribution patterns of the five most frequently occurring species by the occurrence sequence method (Schoener and Schoener 1983). Lakes were ranked along sequences of increasing value for each environmental factor; we then analyzed patterns of occurrence of individual species along these sequences for ordered or haphazard distributions via Mann–Whitney *U*-tests. If presence or absence of a species is related to an environmental factor, then the species' distribution should be concentrated at one end of the environmental sequence; if there is no relationship, then the occurrence distribution should not reveal a pattern relative to the sequence. This analysis determines the precision of any distributional threshold along an environmental gradient without making a priori assumptions about the exact location of such a threshold (Schoener and Schoener 1983; Cornell 1986).

Canonical analysis (Berenson et al. 1983) was used to evaluate the extent to which groups of lakes, as described from classification and occurrence sequence analyses, were environmentally different. All environmental variables used in the analysis, except pH, were normalized by using a $\log_{10}$ transformation. We conservatively estimated group separation by visual inspection (after Green and Vascotto 1978) and by analyzing the percentage of lakes correctly classified into an assemblage group according to the environmental classification functions. Finally, means of each lake group for each environmental variable were tested for significant differences using analysis of variance.

Results

Species Composition and Sampling Efficiency

Overall, 11 fish species were recorded in 36 lakes (Table 1); nine lakes were fishless. In only one case (see below) did we catch too few fish of a species to satisfy our minimum criteria to be included in that lake's assemblage. Of the remaining lakes with fish, the Beaver Lake assemblage consisted of coldwater species only (burbot (*Lota lota*) and rainbow trout (*Salmo gairdneri*)) and was thus considered to be ecologically distinct and excluded from further analyses.

Of the six resampled ponds, four showed no change in the number or composition of species present during 1986 and 1987 (Table 2). In Sara Lake, a total of three Iowa darters (*Etheostoma exile*) were caught only on the second of four sampling dates, and thus were not included as part of the assemblage. Gods Lake showed a doubling of species between May and August 1986, but stabilized in species composition thereafter. This increase in species number was most likely related to unusually high precipitation during July 1986 that opened up an intermittent stream, allowing northern pike and white suckers (*Catostomus commersoni*) to invade. Because both species maintained large populations for over a full year, they were included in the Gods Lake assemblage. Based on the overall results of this resampling program, we concluded that the sampling protocol was consistent and efficient at catching susceptible species, and thus the results adequately represented the composition of the assemblages.

Pattern Analysis

Hierarchical clustering of the 35 lakes with fish distinguished two major assemblage types at the 1% similarity level (Fig. 2). DCA also identified the two main assemblage types but, in addition, identified three lakes that had a mixed species compositon (Fig. 3). Both major assemblage types can be described by their more common and characteristic members: brook stickleback (*Culaea inconstans*) and fathead minnow (*Pimephales promelas*) versus northern pike and yellow perch. White suckers occurred twice as frequently in pike/perch lakes than in stickleback/fathead lakes whereas three additional cyprinid species were restricted to the latter assemblage type (Table 1).

Of the environmental information collected for each lake, we retained maximum depth, surface area, pH, presence of inlet/outlet, water color, vegetation diversity, conductivity, and phosphorus for further analysis (Appendix I). These variables had fewer than three significant intercorrelations and thus provided some independent description of the lake environment. We did not use Secchi depth, elevation, or shoreline length in any of the analyses. Correlation analysis of the remaining eight environmental variables with species richness indicated that only pH had a significant correlation ($r = 0.456$, $P < 0.01$). However, when fishless lakes are dropped from the correlation analysis ($n = 36$), surface area in addition to pH have signif-

TABLE 1. Lakes-by-species (presence/absence) data matrix for the 36 study lakes with fish. Lake ID refers to those used on Fig. 3 and 4. Species belong to a brook stickleback/fathead minnow assemblage (bs/fm), a northern pike/yellow perch assemblage (Pike), or to a mixed species assemblage (Mixed). Lake 10 contains coldwater (cw) species only. bs, brook stickleback (*Culaea inconstans*); fm, fathead minnow (*Pimephales promelas*); np, northern pike (*Esox lucius*); ws, white sucker (*Catostomus commersoni*); yp, yellow perch (*Perca flavescens*); fd, finescale dace (*Phoxinus neogaeus*); rd, northern redbelly dace (*Phoxinus eos*); pd, pearl dace (*Semotilus margarita*); Id, Iowa darter (*Etheostoma exile*); bt, burbot (*Lota lota*); rt, rainbow trout (*Salmo gairdneri*).

				Species											
Lake	ID	Lat.	Long.	bs	fm	np	ws	yp	fd	rd	pd	Id	bt	rt	Assemblage
Mariana	64	55°50	112°06			1									Pike
Unnamed	52	54°32	113°41			1									Pike
Teen	50	54°29	113°43			1									Pike
Unnamed	30	54°14	114°44					1							Pike
Gregg	04	53°39	117°41			1	1								Pike
Unnamed	42	54°25	113°14			1	1								Pike
Unnamed	25	54°19	114°59			1		1							Pike
Jackfish	62	54°45	113°05			1		1							Pike
Banana	55	54°43	113°50			1		1							Pike
Lofty	60	54°43	112°29			1	1	1							Pike
Crooked	54	54°55	113°32			1	1	1							Pike
Ghost	63	54°52	113°35			1	1	1							Pike
Jenkins	61	54°45	113°35			1	1	1				1			Pike
Unnamed	49	54°38	113°35		1										bs/fm
Unnamed	27	54°13	114°45	1											bs/fm
Unnamed	28	54°05	114°42	1	1										bs/fm
Unnamed	29	54°12	114°28	1	1										bs/fm
Unnamed	31	54°06	114°47	1	1										bs/fm
Unnamed	43	54°26	113°08	1	1										bs/fm
Unnamed	45	54°39	113°38	1	1										bs/fm
Unnamed	11	53°31	117°40	1	1										bs/fm
Unnamed	33	54°17	115°03	1	1										bs/fm
Canoe	53	54°37	113°07	1	1										bs/fm
Unnamed	51	54°36	113°12	1	1										bs/fm
Unnamed	57	54°12	113°20	1	1										bs/fm
Spear	48	54°44	113°09	1	1										bs/fm
Unnamed	41	54°41	113°36	1	1		1								bs/fm
Unnamed	44	54°44	113°40	1	1		1								bs/fm
Sara	35	54°37	114°12	1	1				1	1					bs/fm
Petite	03	53°40	117°45	1	1				1	1	1				bs/fm
Unnamed	07	53°16	117°48	1					1	1					bs/fm
Emerson	12	53°44	117°50				1		1		1		1		bs/fm
Gods	47	54°24	113°39	1	1	1	1								Mixed
Marlboro	15	53°33	116°50	1		1	1								Mixed
Duck	56	54°38	113°57	1	1	1	1	1							Mixed
Beaver	10	53°15	117°26										1	1	cw

TABLE 2. Species composition over time in a subset of six lakes where sampling was replicated in May and August 1986 and 1987. Lake names and numbers refer to Table 1. Blanks, no sampling done; bs, brook stickleback; fm, fathead minnow; np, northern pike; ws, white sucker; yp, yellow perch; Id, Iowa darter; d, dace species.

	Sampling date			
Lake	May 1986	Aug. 1986	May 1987	Aug. 1987
43	bs, fm	bs, fm	bs, fm	bs, fm
41	bs, fm, ws	bs, fm, ws	bs, fm, ws	bs, fm, ws
44	bs, fm, ws	bs, fm, ws		bs, fm, ws
Sara	bs, fm, d	bs, fm, d, Id	bs, fm, d	bs, fm, d
Gods	bs, fm	bs, fm, np, ws	bs, fm, np, ws	bs, fm, np, ws
Jenkins	yp, np, ws, Id	yp, np, ws, Id	yp, np, ws, Id	

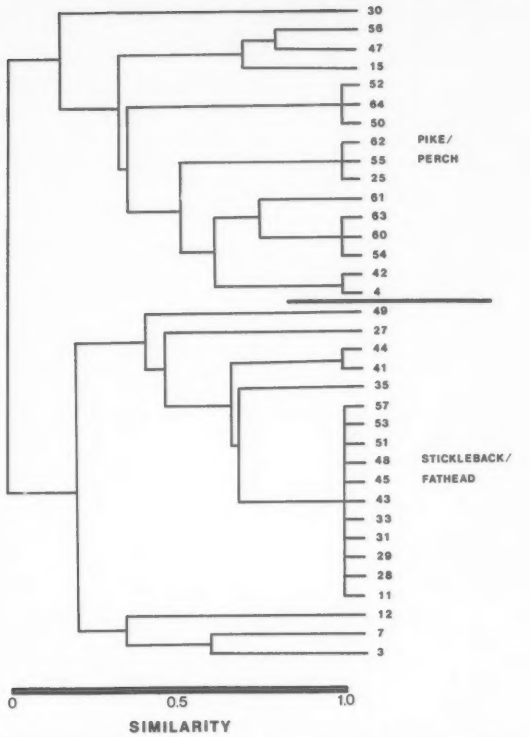

FIG. 2. Hierarchical classification of 35 lakes with fish (excluding Lake 10), using Jaccard's coefficient of similarity and species presence/absence. Numbers refer to lakes listed in Table 1.

icant correlations with species richness ($r = 0.396$, $P < 0.05$ and $r = 0.567$, $P < 0.01$, respectively).

Occurrence sequence analysis indicated that yellow perch were found significantly more often in lakes that were deeper, larger in surface area, and lower in water color than lakes without this species (Table 3). Based on this result, we divided the previously identified pike/perch assemblage into two subassemblages, namely, pike only ($n = 5$) and pike/perch ($n = 8$). Brook stickleback and fathead minnows were found significantly more often in lakes of small surface area. All five common species were distributed independently across gradients of lake isolation, pH, phosphorus concentration, vegetation diversity, and conductivity.

The three lakes with mixed species assemblages (15, 47, 56; Table 1), one lake with coldwater species (10), and three lakes with missing environmental data (51, 57, 100; Appendix I) were excluded from the canonical analysis. The first two canonical variates, which accounted for 76 and 17% of the among-group variance, respectively, separated pike/perch lakes from pike only, stickleback/fathead, and fishless lakes (Fig. 4). Classification of assemblage groups indicated that pike only and stickleback/fathead lakes had the lowest (40 and 60%, respectively), fishless lakes intermediate (75%), and pike/perch the highest (88%) total percent correct classification. The misclassified pike/perch lake was classified as a pike only lake. Pike only, stickleback/fathead, and fishless lakes did not differ significantly from each other with respect to any environmental variable considered individually (Table 4). All three differed significantly from pike/perch lakes with respect to surface area and maximum depth. Overall, results of the canonical analysis indicated considerable environmental similarity among pike only, stickleback/fathead, and fishless lakes whereas pike/perch lakes were environmentally distinct. If stickleback/fathead, pike

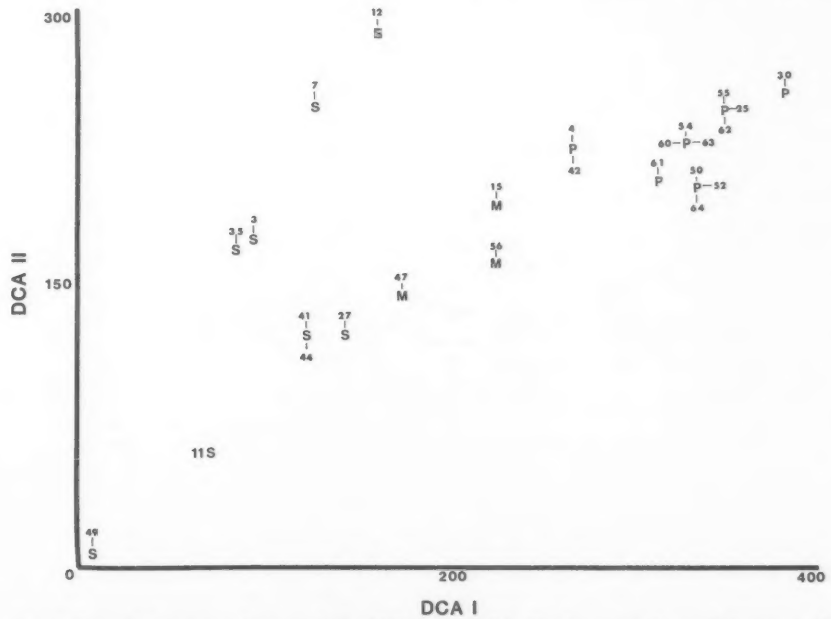

FIG. 3. Detrended correspondence analysis of 35 lakes with fish. Numbers around a symbol correspond to lakes in Table 1; Lake 10 is excluded and 11S refers to Lakes 11, 28, 29, 31, 33, 43, 45, 48, 51, 53, and 57 that had the same DCA score. P, pike/perch assemblage; S, stickleback/fathead assemblage; M, mixed assemblage consisting of species from both S and P.

TABLE 3. Results of occurrence sequence analysis for the five most common fish species along gradients of eight environmental variables. Species are classified as being concentrated at the low or high end of a gradient, or distributed throughout a gradient, based on Mann–Whitney *U*-tests of their patterns of presence and absence.

Environmental variable	Distribution of occurrence		
	Low	Throughout	High
Conductivity		5 species[a]	
Inlet/outlet		5 species	
Maximum depth		4 species	Perch
pH		5 species	
Phosphorus		5 species	
Surface area	bs, fm	2 species	Perch
Vegetation		5 species	
Water color	Perch	4 species	

[a]Brook stickleback (bs), fathead minnow (fm), yellow perch, northern pike, and white sucker.

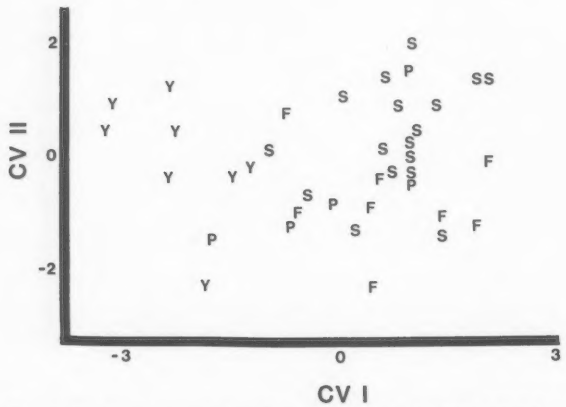

FIG. 4. Graphic representation of a canonical analysis of 38 study lakes using eight environmental variables (see Table 4). Lakes were grouped into assemblage types and fishless lakes based on the results of classification, ordination, and occurrence sequence analyses. Three mixed assemblage lakes (15, 47, 56), one lake with rare species (10), and three lakes lacking complete environmental data (51, 57, 100) were excluded from the canonical analysis. S, stickleback/fathead; Y, pike/perch; P, pike only; F, fishless.

only, and/or fishless assemblages could occur at equal frequencies among the 30 environmentally similar, small, shallow lakes (excluding the eight larger, deeper pike/perch lakes), then there are significantly more stickleback/fathead lakes, significantly fewer pike only lakes, and an expected number of fishless lakes ($G = 7.56$, 2 df, $P < 0.05$).

Discussion

Fish Assemblages of Central Alberta

Average fish species richness of small lakes in central Alberta was relatively low (2.4 species) compared with small lakes of southern Ontario (e.g. 10.6 species, Harvey 1981) and Wisconsin (e.g. 5.3 species, Tonn et al. 1983; 11 species, Rahel 1986). This result was not unexpected because of the great distance from major glacial refugia (e.g. Mississippi River Basin, Lindsey and McPhail 1986) and an increase in climatic severity as one moves north. Despite low species richness, we were able to identify two distinct fish assemblage types, one characterized by brook sticklebacks and fathead minnows and the other by northern pike and yellow perch.

TABLE 4. Mean (and range) of eight environmental variables for each assemblage type and fishless lakes. All variables except pH were $\log_{10}$-transformed prior to statistical analysis. The three lakes with mixed assemblages (15, 47, 56), one lake with coldwater species (10), and three lakes with environmental data missing (50, 57, 100) are excluded. The variables are presented in descending order of *F* values obtained from one-way ANOVAs (*$P < 0.05$; 3,35 df).

	Assemblage type				
	st/fm[a]	Pike only	Pike/perch	Fishless	*F*
No. of lakes	17	5	8	8	
Maximum depth (m)	3.20 (7.50)	4.20 (6.40)	9.00 (10.70)	2.40 (9.0)	7.79*
Surface area (ha)	15.92 (96.25)	19.52 (56.07)	65.63 (66.25)	15.64 (93.75)	4.63*
pH	8.21 (1.80)	7.64 (1.20)	8.38 (1.90)	7.98 (2.30)	1.95
Outlet[b]	0.39 (0.50)	0.52 (2.00)	0.64 (6.00)	0.11 (0.50)	1.73
Water color (mg Pt/L)	22.32 (30.12)	31.85 (57.10)	18.46 (40.00)	37.23 (92.50)	1.05
Vegetation[c]	6.75 (6.00)	6.45 (2.00)	6.28 (4.00)	6.05 (6.00)	0.67
Conductivity (μS/cm)	273.37 (404.00)	244.02 (304.00)	270.89 (154.00)	226.76 (338.00)	0.41
Phosphorus (mg/L)	52.71 (72.40)	47.28 (74.00)	40.13 (155.60)	45.12 (103.00)	0.19

[a]Stickleback/fathead.
[b]0.00, absent; 0.50, intermittent; 1.00, present.
[c]Higher numbers = greater macrophyte diversity/abundance.

The most readily apparent difference between the two main assemblages was the nearly perfect negative association between pike/perch members and most members of the stickleback/fathead assemblage. Piscivores have been noted to alter assemblage composition in small boreal lakes by excluding predation intolerant species (Harvey 1981; Tonn and Magnuson 1982). As in north-central North America, exclusion of species in our lakes was limited to small-bodied cyprinids whereas species with relatively large adult body size (e.g. white suckers) often coexisted with piscivore populations (see also Tonn and Magnuson 1982).

In our study lakes, yellow perch may ultimately rely upon a combination of large adult body size and dorsal spines as mechanisms to increase survival in the presence of northern pike. The presence of spines has been shown to reduce predator effectiveness and thus aid in the coexistence with predators (Hoogland et al. 1957; Reist 1980; Tonn and Magnuson 1982). Even though brook sticklebacks coexisted with northern pike to a greater extent than similarly sized but spineless cyprinids, it is unclear if brook stickleback spines alone provide protection from piscivory. This is particularly dubious in light of the small spines of Alberta brook sticklebacks (Nelson 1969) and the strong interaction between predator/prey size ratios and spine effectiveness (Reist 1980). Overall, increased coexistence with northern pike displayed by brook sticklebacks in our study lakes is most likely a result of combined effects of habitat use, behavioral responses to presence of northern pike (see Reist 1980), and spines.

Mixed assemblages of small-bodied species and piscivores are exceptions in small Alberta lakes and their species composition may reflect temporary conditions of the lake due to historic events. During our study we documented the colonization of Gods Lake by northern pike, presumably via an intermittent inlet. Duck Lake also contained a large population of northern pike but relatively few brook stickleback and fathead minnows. Perhaps predation by recently immigrated northern pike had reduced but not yet eliminated populations of these small-bodied species or the presence of a permanent northern pike population had not allowed for the successful establishment of immigrant populations of brook sticklebacks and fathead minnows. The former event may be more plausible because local residents indicate that pike cannot overwinter in this shallow lake and historically, pike have undergone a spring immigration from and a fall emigration to the nearby Athabasca River.

The occurrence of northern pike, brook sticklebacks, and fathead minnows in small, shallow lakes suggests that these species are able to tolerate severe conditions associated with these lakes such as winterkill. Under natural conditions, these species can avoid conditions of winter hypoxia by emigration (Tonn and Magnuson 1982; Magnuson et al. 1985), altering under-ice distributions (Magnuson and Karlan 1970; Tonn and Paszkowski 1987), and by increasing ventilation rate and reducing movement (Petrosky and Magnuson 1973).

Even though species in pike only and stickleback/fathead assemblages may tolerate or avoid severe conditions of small, shallow lakes, significant differences in relative occurrence of each assemblage type were observed. The low frequency of pike only and the high frequency of stickleback/fathead assemblages may be related to the reproductive requirements of individual species (Scott and Crossman 1973; Rahel 1984). For example, the absence of vegetated bays or marshes and the frequent absence of juvenile northern pike from small, shallow lakes compared with large perch/pike lakes suggests reduced northern pike reproduction. In contrast, we found an abundance of appropriate reproductive habitat in these small, shallow lakes that could allow brook stickleback and fathead minnows to maintain large populations.

The greater than expected occurrence of stickleback/fathead assemblages in small, shallow lakes may also be associated with the lifelong possession of small bodies by brook sticklebacks and fathead minnows that would allow these species to disperse during periods of low surface run-off (Becker 1983). Overall, the combined effects of these and other factors suggest that small, shallow Alberta lakes are more suitable for and available to brook sticklebacks, fathead minnows, and associated species than for northern pike.

Yellow perch, as a member of the pike/perch assemblage, were limited to deeper, larger lakes in the study set. Lakes of this type may have a lower probability of winterkill (Magnuson et al. 1985) and have been associated with a greater abundance of prey and usable habitat (Eadie and Keast 1984). Elsewhere, however, yellow perch are frequently found in small, shallow lakes as well as larger, deeper lakes (Tonn and Magnuson 1982; Rahel 1984). Why are perch largely restricted to larger, deeper lakes in central Alberta?

Infrequent occurrence of yellow perch in small, shallow Alberta lakes may ultimately be related to differences in combined effects of relative density, connectedness, and productivity of these lakes. Southern Ontario and the Northern Highlands district of Wisconsin contain higher densities of lakes, and more of them have inlets/outlets, compared with Alberta. Alberta lakes are also considered to be far more productive than similarly sized lakes in Wisconsin and Ontario because of major differences in phosphorus source and retention (Riley and Prepas 1984). Because oxygen depletion and subsequent fish kills are positively linked with increased productivity (Barica 1975; Babin and Prepas 1985), shallow Alberta lakes may be subjected to more frequent and severe winterkill events than small lakes in north-central North America. High productivity coupled with greater relative isolation of small Alberta lakes may result in higher extinction rates, lower colonization rates, and a reduced occurrence frequency of yellow perch.

Regional Comparisons

The results of our study, combined with those from Wisconsin and Ontario, indicate that despite significant differences among regions, relatively few environmental variables are important in structuring fish assemblages in small north-temperate lakes. In north-central North America, pH, surface area (and its correlate habitat diversity), depth (or winter oxygen concentrations), and lake isolation have frequently been noted as being most important in identifying or predicting lakes containing different types of fish assemblages and/or different levels of species richness (Johnson et al. 1977; Harvey 1975, 1978, 1981; Tonn and Magnuson 1982; Rahel 1984; Eadie and Keast 1984; Eadie et al. 1986). In Alberta, we similarly identified depth and surface area as important environmental factors related to assemblage composition.

In our Alberta lakes, pH ranged from 7.0 to 10.0, well above levels in most shield lakes (but comparable with off-shield lakes in Ontario; Harvey 1978, 1981). For most fishes, pH in this range is not physiologically stressful and should not play a major role in directly influencing assemblage structure. However, for our study lakes, pH was found to have a significant positive correlation with species richnes. This result may be a statistical artifact owing to the small range in species richness (0–5) or to an intercorrelation with surface area, a factor having a significant positive correlation with species richness.

Somewhat more surprising is the relatively low importance in Alberta of factors associated with lake isolation (the presence, number, and type of inlet/outlet). An important difference between central Alberta and other regions where isolation plays a key role in structuring fish assemblages is that a large proportion (70%) of our study lakes had intermittent inlets/outlets. The absence of historical data on flow regimes in these streambeds made it impossible for us to quantify the degree of stream intermittency. As a result, equal treatment of intermittent streams may have masked the true contribution made by lake isolation in structuring Alberta fish assemblages. Based on our observations, e.g. the appearance of northern pike and white suckers in Gods Lake following a period of heavy rainfall, it seems likely that colonization or periodic recolonization of species can also have important consequences to assemblage structure in small Alberta lakes.

In contrast with these environmental factors, piscivory appeared to be at least as important in structuring fish assemblages in Alberta as in north-central North America. The mutually exclusive or nearly exclusive cooccurrence patterns of northern pike and several smaller species were consistent with patterns in other regions and points to a widespread influence of the impact of predation on small-lake assemblage compo-

sition. We suggest that the small population sizes and/or the simple habitat structure common in small lakes, combined with the presence of small, vulnerable species, favour the occurrence of strong, direct, assemblage-level effects of piscivory in these systems.

Conclusions

Our analyses support and extend results from other regions in demonstrating that piscivory and aspects related to environmental severity (maximum depth and winterkill), habitat heterogeneity (surface area), and, to a lesser extent, isolation (presence and type of inlet/outlet) have widespread influence in structuring fish assemblages in small north-temperate lakes. Identification of a small number of local environmental factors having widespread influence on fish assemblages may be, in part, due to inherent characteristics associated with the discrete, insular, and structurally simple nature of small lakes and the limited response of small lakes to common aspects of the north-temperate climate. In contrast, regional factors such as edaphic influences on productivity and water chemistry (pH) and the relative isolation of Alberta from glacial refugia may have minimized the richness of individual assemblages, the number of distinct assemblage types, and/or the discriminatory power of our analyses, relative to other regions of North America. Our study, based in a region of northwestern North America hundreds of kilometres from where previous studies of fish assemblages in small lakes have been concentrated, clearly reveals that the structures of these assemblages bear the imprints of both local and regional influences.

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APPENDIX 1. Morphometric and limnological characteristics of the 45 lakes sampled within the Athabasca River basin. Fishless lakes are numbered 100 – 108. For remaining lakes, refer to Table 1 for species composition and lake name. Blanks indicate no data collected.

ID	pH	Color (mg Pt/L)	Max. *z* (m)	Conductivity (μS/cm)	Surface area (ha)	Vegetation[a]	Phosphorus (mg/L)	Outlet[b]
3	8.20	002.50	04.00	197.00	011.25	05.00	013.90	0.00
4	7.90	012.50	04.50	321.00	006.43	07.00	026.00	2.00
7	8.70	010.00	03.00	458.00	010.83	06.00	021.30	0.50
10	7.80	040.00	01.60	240.00	003.75	07.00	018.60	0.50
11	8.00	050.00	01.50	255.00	003.75	05.00	019.10	0.50
12	7.80	050.00	07.25	317.00	007.50	06.00	016.30	0.50
15	8.12	020.00	03.50	207.00	100.00	07.00	018.50	0.50
25	7.30	010.00	07.00	300.00	051.25	07.00	050.00	0.50
27	7.50	006.00	02.00	282.00	007.50	07.00	058.00	0.50
28	7.60	015.00	02.75	484.00	025.00	08.00	045.00	0.50
29	8.30	030.00	03.00	370.00	030.20	07.00	065.00	0.50
30	8.05	015.00	07.00	364.00	028.75	06.00	024.00	0.50
31	8.10	020.00	03.00	380.00	024.17	08.00	030.00	0.50
33	8.00	010.00	07.00	415.00	043.33	06.00	040.00	0.50
35	8.50	010.00	06.00	215.00	090.00	08.00	029.30	0.50
41	8.10	030.00	09.00	330.00	007.50	07.00	036.50	0.50
42	7.70	050.00	02.00	400.00	012.00	08.00	100.00	2.00
43	8.15	040.00	01.75	236.00	018.00	11.00	086.30	0.50
44	9.10	100.00	01.50	080.00	029.20	06.00	080.00	0.50
45	7.70	070.00	02.70	190.00	010.00	06.00	360.00	0.50
47	8.30	020.00	04.50	268.00	100.00	06.00	043.00	0.50
48	9.00	007.50	02.00	174.00	010.00	06.00	041.10	0.50
49	7.50	100.00	04.60	248.00	003.75	07.00	074.00	0.50
50	7.80	025.00	08.40	270.00	036.25	07.00	028.00	0.00
51	8.10	040.00	01.50		014.00		070.00	0.50
52	8.00	070.00	04.00	260.00	016.25	06.00	048.00	0.50
53	9.30	040.00	02.40	417.00	100.00	08.00	500.00	0.50
54	9.20	050.00	07.40	260.00	080.00	09.00	057.00	6.00
55	8.10	010.00	16.70	212.00	057.50	06.00	016.40	2.00
56	9.85	030.00	01.60	158.00	100.00	08.00	050.10	4.00
57			02.50		062.50	08.00		0.50
60	8.60	030.00	07.00	300.00	070.00	05.00	172.00	0.50
61	9.00	015.00	13.00	261.00	090.00	06.00	026.00	4.00
62	8.20	020.00	06.00	210.00	085.00	06.00	021.40	0.00
63	8.60	020.00	13.00	293.00	095.00	06.00	049.00	0.50
64	6.80	030.00	04.50	096.00	062.50	06.00	060.00	2.00
100	8.10		01.75	235.00	002.50	05.00		0.50
101	8.30	007.50	01.75	254.00	100.00	06.00	020.40	0.50
102	7.50	070.00	01.75	115.00	002.50	04.00	015.90	0.00
103	8.00	055.00	02.50	315.00	010.00	06.00	119.00	0.00
104	8.20	020.00	06.75	289.00	020.00	06.00	036.00	0.00
105	7.90	100.00	01.00	139.00	042.50	07.00	065.00	0.50
106	6.80	040.00	02.00	157.00	042.50	10.00	040.00	0.50
107	8.10	020.00	10.00	453.0	006.25	06.00	045.00	0.50
108	9.10	080.00	01.00	266.00	006.25	05.00	085.00	0.50

[a]Higher numbers = greater macrophyte diversity/abundance.
[b]0.00, absent; 0.50, intermittent; *x*/2, number of outlets.

Phytoplankton, Nutrients, and Primary Production in Fertilized and Natural Lakes at Saqvaqjuac, N.W.T.

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Whole-lake phosphorus (P) and nitrogen (N) addition experiments at Saqvaqjuac, N.W.T. (63°N in the central Canadian arctic), showed that the lakes were P limited but required both P and N for increased production. Photosynthetic response to 0.1 g P and 1.0 g $N \cdot m^{-2} \cdot yr^{-1}$ was immediate (15⟶30 g $C \cdot m^{-2} \cdot yr^{-1}$), with simultaneous increases in protozoa, while oligotrophic chrysophyte assemblages gave way to volvocalean greens. Cyanophytes were not important during P-only or P and N additions or in oligotrophic lakes, but formed permanent blooms in several naturally mesotrophic lakes near sea level. Retention of P was naturally low, but high during P addition. Silicon (Si) retention was always very high. Chlorophyll:P ratios were similar to those of subarctic and north-temperate lakes. Saturation light intensity (I_k) tracked surface light flux with a 2- to 3-wk delay, averaging 15 $E \cdot m^{-2} \cdot s^{-1}$ in winter and peaking at 140 $E \cdot m^{-2} \cdot s^{-1}$ in July. Comparison of our data with those for lakes in the Experimental Lakes Area (northwestern Ontario, 50°N) and Char Lake (75°N) shows that with increasing latitude, in small lakes, (a) phytoplankton production decreases, (b) phytoplankton production per unit light decreases less sharply, and (c) the proportion of primary production occurring beneath ice cover increases.

Les essais d'addition de phosphore (P) et de azote (N) sur toute la superficie des lacs à Saqvaqjuac, N.W.T. (située à 63° de latitude Nord dans le centre de l'Arctique canadien), ont révélé que la croissance dans les lacs était limitée par la teneur en P, mais que l'addition de P et de N était nécessaire pour en accroître la production. L'effet de l'addition de 0,1 g de P et de 1,0 g de $N \cdot m^{-2} \cdot an^{-1}$ sur la production photosynthétique a été immédiate (15⟶30 g de $C \cdot m^{-2} \cdot an^{-1}$); on a observé parallèlement des augmentations du nombre de protozoaires, tandis que les groupements de chrysophycées oligotrophes ont été déplacés par des volvocidés. Les cyanophycées n'étaient abondantes ni après l'addition de P ou de P et de N ni dans les lacs oligotrophes, mais formaient en permanence des colonies apparentes dans plusieurs lacs naturellement mésotrophes situées près du niveau de la mer. La rétention du P était naturellement faible, mais elle est devenue élevée au moment de l'addition de cette substance. La rétention de silicium (Si) est demeurée invariablement très élevée. Les rapports chlorophylle:P étaient similaires aux rapports des lacs subarctiques et de ceux du nord de la zone tempérée. Les valeurs d'intensité de saturation lumineuse (I_k) suivaient le flux lumineux avec un décalage de 2- à 3-sem, atteignant une moyenne de 15 $E \cdot m^{-2} \cdot s^{-1}$ en hiver et une pointe de 140 $E \cdot m^{-2} \cdot s^{-1}$ en juillet. En comparant nos données à celles relevées dans la région des lacs expérimentaux (50° de latitude Nord) et le lac Char (75° de latitude Nord), on constate que dans les petits lacs, la production de phytoplancton diminue, la production de phytoplancton par unité d'intensité lumineuse diminue de façon moins marquée et la production primaire sous la couverture de glace augmente, au fur et à mesure qu'on se déplace vers le nord.

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In the last two decades there has been a great deal of work on nutrient control of lake productivity. Under oligotrophic to mesotrophic conditions, phosphorus (P) has been shown to exert overall control, both on phytoplankton biomass (Vollenweider 1968; Dillon and Rigler 1974, and others) and productivity (Schindler et al. 1978). Nitrogen (N) becomes a colimiter as the N:P ratio in nutrient supply decreases below about 25:1 by weight (Smith 1982). If excess P is present (usually as a result of domestic or industrial waste discharged to the water body), blooms of N_2-fixing Cyanophyta (blue-green algae) frequently result (Schindler 1977). Carbon (C) may temporarily limit daily production in low-carbonate, nutrient-enriched waters (Schindler 1977), but long-term control is exerted by N and P. Much of the experimental evidence for these effects was obtained during whole-lake manipulations at the Experimental Lakes Area (ELA), northwestern Ontario (49°40′N), (Schindler 1980).

Relatively little work has been done to verify these temperate zone results for the enormous number of arctic lakes. Work at ultraoligotrophic Char Lake and nearby sewage-enriched Meretta Lake (74°42′N) in the Canadian high arctic showed that phytoplankton production was very low when N and P were in short supply (Kalff and Welch 1974; Kalff et al. 1975) whereas chlorophyll increased 10- to 100-fold in response to domestic sewage pollution. Shallow (0.2 m) tundra ponds at Barrow, Alaska (~71°N), are normally P limited (Stanley and Daley 1976). Deeper ponds (1.2–1.6 m mean depth) in the Arctic Foothills of Alaska (65°N) respond quickly to P addition, but require additional N as well if the P is to be fully utilized (McCoy 1983). Studies at several low-arctic/boreal forest sites have verified that oligotrophic northern lakes are P limited but

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TABLE 1. Morphometry of selected headwater Saqvaqjuac lakes.

Lake	Z_{mean}[a]	Z_{max}	A_0[b] (ha)	A_D[c] (ha)	$A_D : A_0$	% ice volume[d]	Elevation (m above msl)[e]
Jade	1.8	5.2	3.63	27.9	7.7	77	31
Far	3.6	8.9	3.70	16.6	4.5	46	18
P + N	3.3	10.2	7.09	28.6	4.0	47	16
Spring	2.7	7.1	6.93	30.6	4.4	56	3

[a]Mean lake depth.
[b]Lake area.
[c]Drainage area excluding lake area.
[d]At 2 m ice thickness.
[e]Far and P + N lakes accurately surveyed; Spring Lake approx. ±1 m; Jade Lake ±5 m.

respond to added P only when N is increased also (Persson et al. 1975; Smith et al. 1984; Holmgren 1985; Granberg 1973). Thus, arctic lakes appear to be nutrient limited in a way similar to temperate lakes.

To quantify the response of arctic lakes to natural and artificially enhanced nutrient loading, we performed a whole-lake nutrient addition experiment at Saqvaqjuac, N.W.T., about 36 km north of the hamlet of Chesterfield Inlet, at 63°39′N on the northwest coast of Hudson Bay. The site was chosen partly because it was geologically similar to ELA, with permafrost instead of trees. Background data and a detailed site description are given in Welch (1985). Additional data are given in Welch and Legault (1986) on chemical limnology and in Welch and Bergmann (1985), Bergmann and Welch (1985), and Welch et al. (1987) on physical limnology. In the present paper we discuss the results of our phytoplankton photosynthesis and dynamics studies under natural and eutrophic conditions at Saqvaqjuac and compare them with the results of other studies, particularly those at ELA. This is the first time that purely latitudinal effects on primary production and nutrients have been assessed with the same methods and on lakes in similar geologic settings.

Methods

Description of the Study Area

The Saqvaqjuac site is on granitic Precambrian Shield rock, glacially scoured, with local relief of 10–30 m. Exposed hilltops are largely bare rock whereas the valleys are covered with coarse glacial till. Vegetation is ericaceous tundra with occasional patches of willow less than 1 m high in wet, protected sites (Welch 1985). Lakes occupy 17% of the land surface area and range from less than 1 ha to 70 km² in area. Natural chemical runoff from the land is low, typically 15–30 g total phosphorus (TP)·ha^{-1}·yr^{-1} and 0.7–1.4 kg total (nongaseous) nitrogen (TN)·ha^{-1}, with electrical conductance of ~50 μS·cm^{-2} (Welch and Legault 1986).

The lakes discussed here are all small headwater lakes 2 km or less from Saqvaqjuac Inlet, a protected arm of Hudson Bay (see Welch 1985 for maps). Some morphometric variables are given in Table 1. Natural nutrient loading is low, ~0.015 g P and ~0.5 g N·m^{-2}·yr^{-1}. Typical concentrations in lakes under nonfertilization conditions are 4–10 mg P and 250–350 mg N·m^{-3}, resulting in 1–2 mg chlorophyll *a* (Chl*a*)·m^{-3} concentrations during open water (Table 2). These small lakes freeze in late September, develop 1.7–2.2 m of ice by April, begin to thaw around 1 June, and become ice-free between 26 June and 21 July (but typically 2–5 July). Water temperatures reach 13–14°C and permanent stratification does not occur (Welch et al. 1987).

After 2 yr of background data collection, annual nutrient addition began in 1979, always in 10 equal weekly allotments beginning at ice-out. P + N Lake received 0.1 g P (as H_3PO_4) and 1.0 g N (as NO_3)·m^{-2}·yr^{-1}, Far Lake received 0.1 g P·m^{-2}·yr^{-1} only, Jade Lake received 0.2 g P and 2.0 g N·m^{-2}·yr^{-1} during 1979 and 1980 only, and Spring Lake was used as a reference system, receiving no added nutrients (Table 2).

TABLE 2. P, N, Chl *a*, and water data for Saqvaqjuac lakes. Q_s = Lake discharge ÷ lake area, except for 1978 P + N and Far, where Q_s = lake inflow ÷ lake area.

Lake	Year[a]	TN supply (kg·ha^{-1})	TN outflow (kg·ha^{-1})	TN retention (kg·ha^{-1})	Q_s	TP supply (kg·ha^{-1})	TP outflow (kg·ha^{-1})	TP retention (kg·ha^{-1})	TP mean open water (mg·m^{-3})	TN mean open water (mg·m^{-3})	Chl *a* mean open water (mg·m^{-3})	N:P mean open water
P + N	1977	—	—	—	—	—	—	—	5.19	275	1.84	53
	1978	5.23	—	—	1.39	0.154	—	—	—	278	1.15	69
	1979[b]	16.06	2.19	0.86	0.60	1.082	0.050	0.95	5.92	363	7.23	61
	1980[b]	16.92	5.99	0.65	1.30	1.126	0.117	0.90	10.24	401	8.22	39
	1981[b]	15.56	3.48	0.78	0.95	1.109	0.079	0.93	12.17	429	8.99	35
Far	1977	—	—	—	—	—	—	—	7.21	265	1.13	37
	1978	5.81	—	—	1.53	0.280	—	—	—	243	1.02	—
	1979[b]	2.95	2.00	0.32	0.70	1.100	0.056	0.95	3.98	236	1.07	59
	1980[b]	7.92	4.86	0.39	2.01	1.135	0.131	0.92	7.83	265	0.98	34
	1981[b]	6.51	7.43	−0.14	1.67	1.184	0.234	0.80	10.75	301	1.46	28
Jade	1979	—	—	—	—	—	—	—	7.11	403	2.77	117
	1980[b]	31.4	9.76	0.69	2.30	2.157	0.084	0.96	15.09	474	2.50	31
	1981	7.80	7.14	0.08	2.06	0.188	0.205	−0.09	10.22	313	3.14	29
Spring	1979	4.23	3.79	0.10	1.24	0.078	0.063	0.19	4.11	338	1.79	82
	1980	9.00	6.74	0.25	2.21	0.141	0.125	0.12	7.81	351	2.85	45
	1981	6.84	8.27	−0.21	1.90	0.140	0.122	−0.15	9.76	375	2.67	38
											Overall mean	50

[a]1977 and 1978, prefertilization years.
[b]Year in which fertilization occurred.

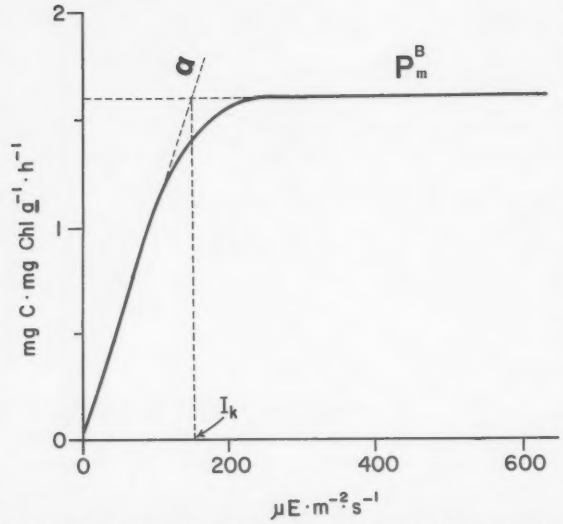


FIG. 1. Parameterization of the incubator-determined photosynthesis per unit Chl *a* (*P*) – irradiance (*I*) response curve. P_m^B is the maximum rate of C fixation (mg C·mg Chl a^{-1}·h^{-1}), α is the initial slope of the function (considered linear through the lowest two irradiances in the incubator) (mg C·mg Chl a^{-1}·E^{-1}·m^{-2}), and I_k is the interception of α with P_m^B. I_k is taken to be the nominal irradiance at the onset of light saturation. The solid curve is the function $P = P_m^B \times \tanh(\alpha I/P_m^B)$ of Platt and Jasby (1976), which fits most of our data well.

Sampling Procedures and Processing

The lakes were usually sampled at weekly intervals June–September 1977–81 and biweekly during the winters of 1977–78 and 1980–81. Four depths were sampled during ice cover and two depths during the open water season, when the lakes were not chemically stratified. Concentrations of Chl *a*, P, N, Si, dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), conductivity, pH, Cl, SO_4, Ca, Mg, K, Na, Fe, and Mn were determined as per Stainton et al. (1977), as discussed in Welch and Legault (1986). Here we deal only with N, P, and soluble-reactive silica (Si). Phytoplankton and protozoans were enumerated from 125-mL subsamples of water bottle casts made to collect primary production material (see below). These subsamples were immediately fixed with acid Lugol's and subsequently preserved in 2% formalin. Aliquots were settled in 10-mL chambers and counts were made with a Wild M40 inverted microscope using the Utermohl method as modified by Nauwerck (1963) and described by Rott (1981). Biomass calculations were based on measurements of linear dimensions using appropriate volume formulae and assuming a specific gravity of 1. Due to difficulty with the taxonomy of many naked chrysophytes and dinoflagellates, our analysis has concentrated on changes in groups of algae and protozoa rather than on individual species and their occurrence. Electron microscopy (scanning (SEM) and transmission (TEM)) was used to identify and record as many of the loricate and scaled chrysophytes, thecate dinoflagellates, and diatoms as possible.

The general approach we used to estimate primary production was to make weekly determinations of the ^{14}C uptake response by phytoplankton as a function of light intensity (so-called *P/I* curves, Fig. 1) and to integrate these with light at depth to give daily photosynthesis estimates (Fee 1984). A vertically integrated 2-L water sample (Shearer 1978) was taken at the deepest point in the lake and processed in the laboratory within 1 h. Under subdued light, DIC, phytoplankton, and Chl *a* samples were removed. Ten 60-mL glass bottles were filled, a volume of water equal to that of the glass stopper plus 0.5 mL removed, and 0.5 mL of $Na^{14}CO_3$ solution added. Four pairs of bottles were placed in a four-level light incubator similar to that of Shearer (1976) and the fifth pair was wrapped in aluminum foil to determine dark uptake. All bottles were incubated at the in situ lake temperature. After a 4-h incubation, bottles were shaken, 8 mL was injected into scintillation vials containing 0.1 mL of 0.1 N HCl, and the vials were bubbled for 20 min (Shearer et al. 1985). Samples were fixed with 100 μL of NCS (Beckman Tissue Solubilizer) and 10 mL of fluor (Beckman Redi-Solv MP). Radioactivity was assayed in a Beckman LS100 liquid scintillation counter. Light levels in the incubator were measured with a LICOR flat-plate quantum collector (LI-190S), corrected as per Fee et al. (1987).

To generate seasonal lake-wide estimates of photosynthesis, Fee's (1984) programs were used. Light input into the model consisted of continuous (5-min integration intervals) light at each metre of depth throughout the year, whenever Saqvaqjuac was occupied, and took into account reflectance (albedo) and transmission through snow, clear ice, candled ice, and water (see Welch et al. 1987 for details). Fee's (1984) model uses empirical data from the *P/I* curves and linearly interpolates between light levels. The model ignores photoinhibition, i.e. it assumes that production at light levels higher than the maximum incubator irradiance is the same as the maximum rate measured in the incubator. There was usually no evidence of photoinhibition at in situ light levels. Linear interpolation between sampling dates was used. See Fig. 1 for definitions of the photosynthetic parameters α, P_m^B, *and* I_k as they are used in this paper.

Results

Phytoplankton in Oligotrophic Lakes

Unfertilized Saqvaqjuac lakes generally had maximum summer biomass <1 g·m^{-3} (Fig. 2 and 3 and unpublished 1976 survey data), classifying them as typically oligotrophic (Vollenweider 1968). Oligotrophic Saqvaqjuac lakes were dominated by Chrysophyta, with dinoflagellates second in importance and cryptophytes third. Chlorophyta had low biomass but very high diversity. Cyanophyta had very low biomass throughout the year. Euglenophytes were usually negligible. Table 3 lists the most abundant species in each group.

According to Holmgren (1983), there are four identifiable phytoplankton assemblages in unpolluted arctic and subarctic lakes: (1) Chrysophyceae, (2) Chrysophyceae–Diatom, (3) Chrysophyceae–Cryptophyceae, and (4) Chrysophyceae–Dinophyceae. The unfertilized oligotrophic lakes of the Saqvaqjuac area in general have the type one Chrysophyceae assemblage. Spring Lake is an exception and at times it fits the type 4 Chrysophyceae–Dinophyceae assemblage, perhaps because it is near sea level and recently isolated from the sea (see Results and Discussion).

Maximum phytoplankton biomass usually occurred in July–August, although an exception occurred beneath the ice of Far and P + N lakes in late April – early May 1978, well prior to

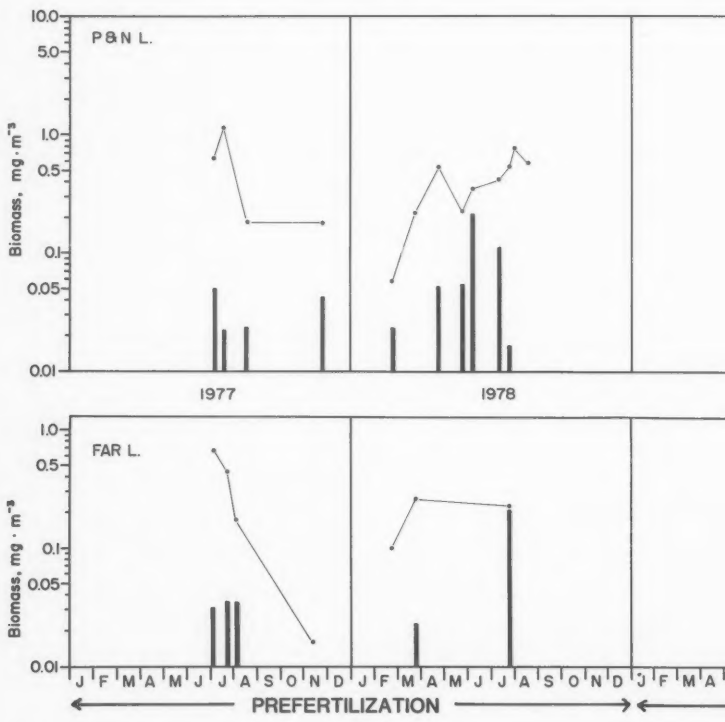
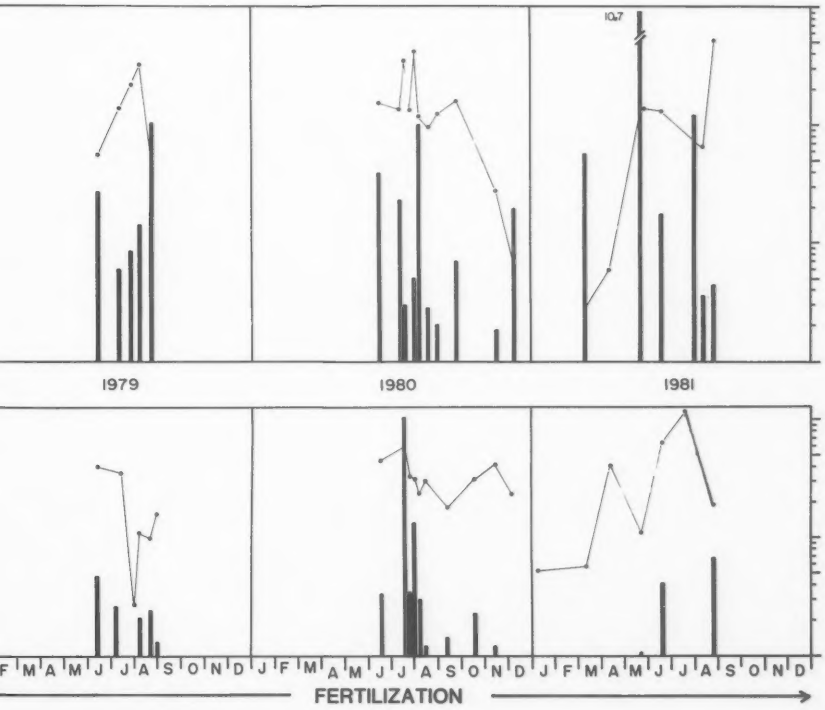


FIG. 2. Biomass of phytoplankton (lines) and Protozoa (bars) in P + N and Far lakes. Wh
Lake was fertilized with P and N during 1979–81. Far Lake received P only at the same ti



kes. Where phytoplankton but not Protozoa are shown, Protozoa biomass was <0.01 mg·m^{-3}. P + N
same time.

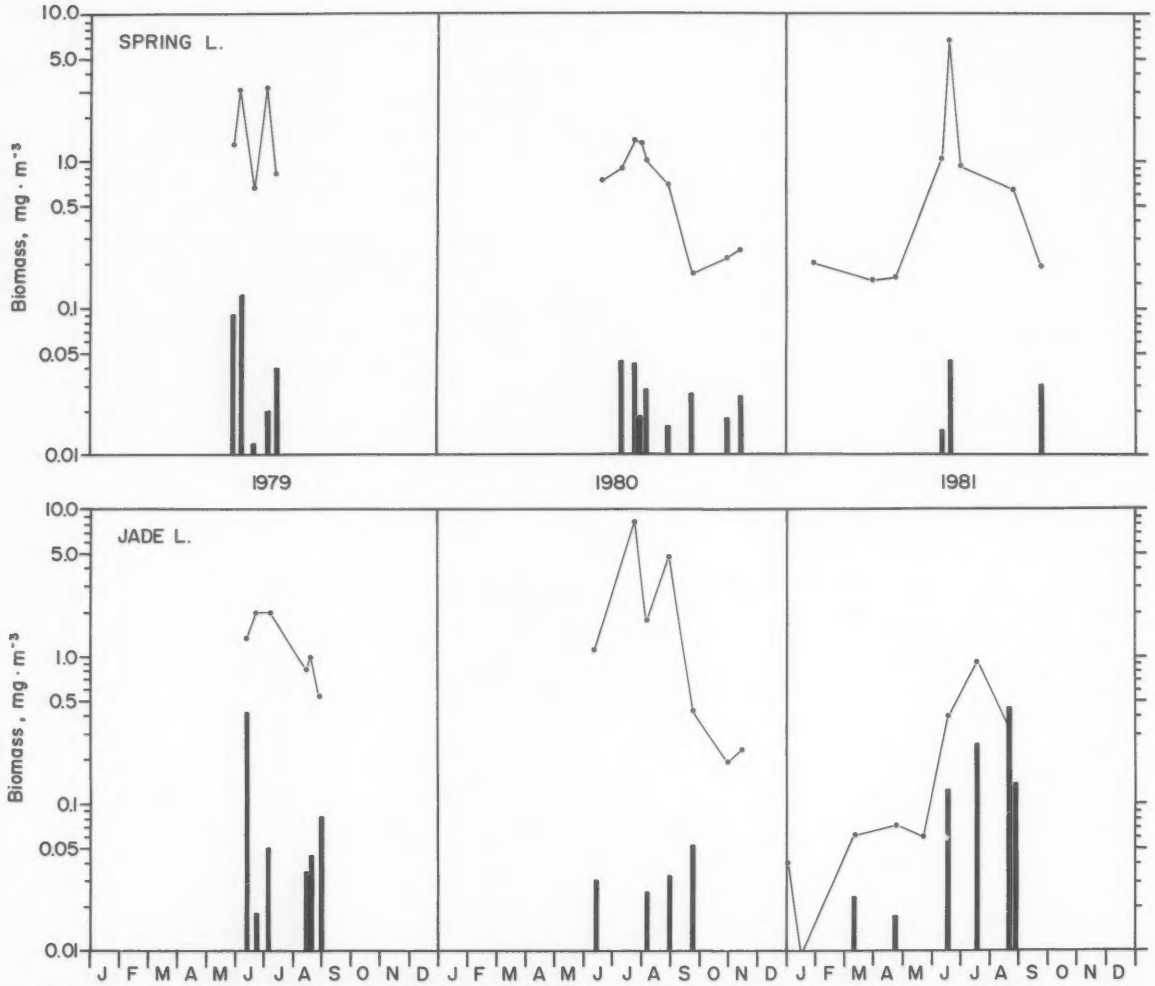


FIG. 3. Biomass of phytoplankton (lines) and Protozoa (bars) in Spring and Jade lakes. Where phytoplankton but not Protozoa are shown, Protozoa biomass was <0.01 mg·m^{-3}. Spring lake was undisturbed. Jade was fertilized with P and N during 1979–80.

spring melt, when biomass reached 0.5 g·m^{-3} in P + N Lake (Fig. 2) and over 7 mg Chl *a*·m^{-3} (see below). That bloom waned in late May and June, and was composed chiefly of the chrysophytes *Ochromonas* spp., *Dinobryon sertularia, D. suecicum, D. attenuatum*, and the dinoflagellates *Gymnodinium uberrimum* and *Peridinium aciculiferum*, plus cryptophytes *Cryptomonas rostratiformis* and *Rhodomonas lacustris* (*minuta* and var. *nannoplanctica*). Since the mean light levels penetrating the water were 4–5 E·m^{-2}·d^{-1} at this time (compared with ~22 E·m^{-2}·d^{-1} during mid-July), those species were either adapted to low light or were heterotrophic, probably the former. The only other under-ice bloom occurred in November 1980 in Far Lake and was composed of diatoms (see below).

Phytoplankton in Fertilized Lakes

After fertilization in P + N Lake there was a rapid and sustained fourfold increase in maximum summer biomass. Responding species are given in Table 4. Chrysophytes dominated in 1979, the first year of fertilization, chrysophytes followed by cryptophytes and dinoflagellates in 1980, and a diversity of groups in 1981, culminating in 5 g *Paulschulzia pseudovolvox* (Chlorophyta)·m^{-3} in late summer of 1981 (Fig. 2). Thus, 3 yr was not enough time for establishment of a stable fertilized phytoplankton community (see Results and Discussion).

Prior to fertilization, Far Lake had a single biomass maximum of 500 μg·L^{-1} during the ice-free season. In 1979, during the first year of fertilization, the total biomass remained less that 0.4 mg·m^{-3}. The pattern was bimodal with a peak in June and another in August. Peridineae and chrysophytes (see Table 5) dominated in June, with the cryptophyte *R. lacustris* (mainly forms *minuta* and *nannoplanctica*) dominant in August. A similar pattern occurred in June–August 1980 although the maximum biomass was 600 μg·L^{-1} in July. Far Lake was the only lake of the four that showed a winter peak, in November 1980. It was composed almost entirely of the diatom *Asterionella formosa* with a slight peak in the dinoflagellate *P. willei*.

TABLE 3. General overview of taxa dominating in oligotrophic Saqvaqjuac lakes.

1. Chrysophyceae	2. Dinoflagellata	3. Cryptophyceae
Dinobyron spp.	*Glenodinium pseudostigmosum*	*Crystomonas reflexa*
Uroglena spp.	*Gymnodinium spp.*	*Crystomonas marssonii*
Chrysochromulina parva	*Peridinium bipes*	*Crystomonas rostratiformis*
Chrysococcus punctiformis	*Perdinium palatinum*	*Rhodomonos lacustris*
Chryosococcus cordiformis	*Peridinium Willei*	*Rhodomonos minuta*
Chrysolykos skuja		*Rhodomonos minuta var. nannoplanctica*
Chrysolykos planctonica		
Ochromonos spp.		
Mallomonas spp.		
4. Bacillariophyceae	5. Chlorophyta	6. Cyanophyta
Asterionella formosa	*Paulschulzia pseudovolvox*	*Gomphosphaeria lacustris*
Tabellaria flocculosa	*Pseudosphaerocystis lacustris*	*Chroococcus limneticus*
Tabellaria fenestrata	*Planctosphaeria gelatinosa*	*Aphanotheca spp.*
Diatoma elongatum	*Teilingia granulata*	*Aphanocapsa spp.*
Cyclotella bodanica var. affinis	*Spondylosium planum*	*Synechococcus spp.*
	Elakatothrix genevensis	*Anabaena spp.*
	Elakatothrix biplex	
	Monoraphidium spp.	

TABLE 4. Phytoplankton species which underwent major (>100 $\mu g \cdot L^{-1}$) changes in abundance during one or more sampling times in P + N Lake (fertilized 1979–81). P = present in quantities sufficient to count ($\gtrsim$ 190 ind.$\cdot L^{-1}$); + = increase; − = decrease.

Species	1977	1978	1979	1980	1981
Asterionella formosa	P	P	+	−	−
Chrysochromulina parva	P	+	P	P	P
Dinobryon bavaricum	P	P	+	+	−
Dinobryon sociale	P	P	P	+	−
Epiphyxis spp.	P	P	P	+	
Gymnodinium sp. 1	P	P	P	+	P
Gymdodinium sp. 2	P	+	P	P	+
Large chrysophytes	P	+	P	+	P
Uroglena americana	P	P	+	+	
Synura sp. 1 + 2	P	P	P	+	
Mallomonas spp.	P	P	P	+	
Paulschulzia pseudovolvox				P	+
Peridinium aciculiferum		P	+	+	−
Peridinium bipes		P	P	+	
Peridinium willei	P	P	P	+	−
Pseudosphaerocystis lacustris			+	P	P
Rhodomonas lacustris		P	P	+	+
Rhodomonas minuta	P	P	P	+	+
Cryptomonas reflexa	P	P	P	+	P
Cryptomonas erosa	P	P	P	P	+
Cryptomonas rostratiformis	P	P	P	+	P
Tabellaria cf. *intermedia*	P		+	P	P

TABLE 5. Phytoplankton species which underwent major (>50 $\mu g \cdot L^{-1}$) changes in abundance during one or more sampling times in Far Lake (P addition 1979–81). P = present in quantities sufficient to count ($\gtrsim$ 190 ind. $\cdot L^{-1}$); + = increase; − = decrease.

Species	1977	1978	1979	1980	1981
Asterionella formosa	P		P	+	P
Chrysochromulina parva			P	+	+
Cyclotella spp.	P	P	P	P	−
Dinobryon cylindricum	P	P	P	+	−
Dinobryon sociale	P		P	+	+
Large chrysophytes	P	P	−	−	−
Mallomonas akrokomos	P		P	+	P
Mallomonas spp.	P	P	P	P	+
Peridinium bipes		P	+	P	P
Peridinium willei	P	+	P	P	P
Rhodomonas lens		P	P		±
Rhodomonas lacustris		P	P	+	+
Rhodomonas minuta	P	P	+	P	P
Rhodomonas minuta var *nannoplanctica*	P	P	P	P	+
Cryptomonas reflexa	P	P	P	P	+
Cryptomonas marssonii	P	P	P	P	+

At this latitude there is sufficient light (10–20 $E \cdot m^{-2} \cdot d^{-1}$; Welch et al. 1987) entering the lakes at that time to allow significant photosynthesis.

In 1981 the pattern changed to more or less one maximum (1200 $\mu g \cdot L^{-1}$) in July, still dominated by chrysophytes (see Table 5). The dinoflagellate peak, approximately of the same magnitude and species of previous years, occurred in June. The population of cryptophytes doubled with *R. lacustris* (*nannoplanctica*) dominating.

Cyanophytes were insignificant in terms of biomass. The difference in cyanophytes in Far Lake compared with those from the other lakes was that, in addition to the genera common to all lakes, the genus *Anabaena* (an N_2 fixer) was present. The percentage of N_2 fixers found in the plankton of this lake was higher than in the other lakes although the biomass was not significantly different. N_2 fixation in the benthos of this lake also increased compared with other lakes (M. Bergmann, Freshwater Institute, pers. comm.).

Jake Lake had very high maximum biomass levels during fertilization, dropping to <1 $g \cdot m^{-3}$ after fertilization in 1981. Cyanophyte biomass was always very low, even though the ratio of N:P was quite low (10:1). Species assemblages were again variable during fertilization.

Protozoa

In unfertilized Saqvaqjuac lakes, small Protozoa (oligotrichs, scutico-ciliates) dominated whereas after increased primary production, much larger species of Holophyridae, Enchylidae, and Paramacidae became abundant. Densities and biomass responded quickly and positively to eutrophication (Fig. 2 and 3). Protozoan biomass averaged 56 $mg \cdot m^{-3}$ in Far Lake during 1977–81 and 31 $mg \cdot m^{-3}$ in Spring Lake during 1979–81, but 92 $mg \cdot m^{-3}$ in Jake Lake during 1979–81. It was 34 and 88 $mg \cdot m^{-3}$ in P + N Lake during 1977–78, but rose rapidly to 569, 791, and 1909 during 1979–81 after fertiliza-

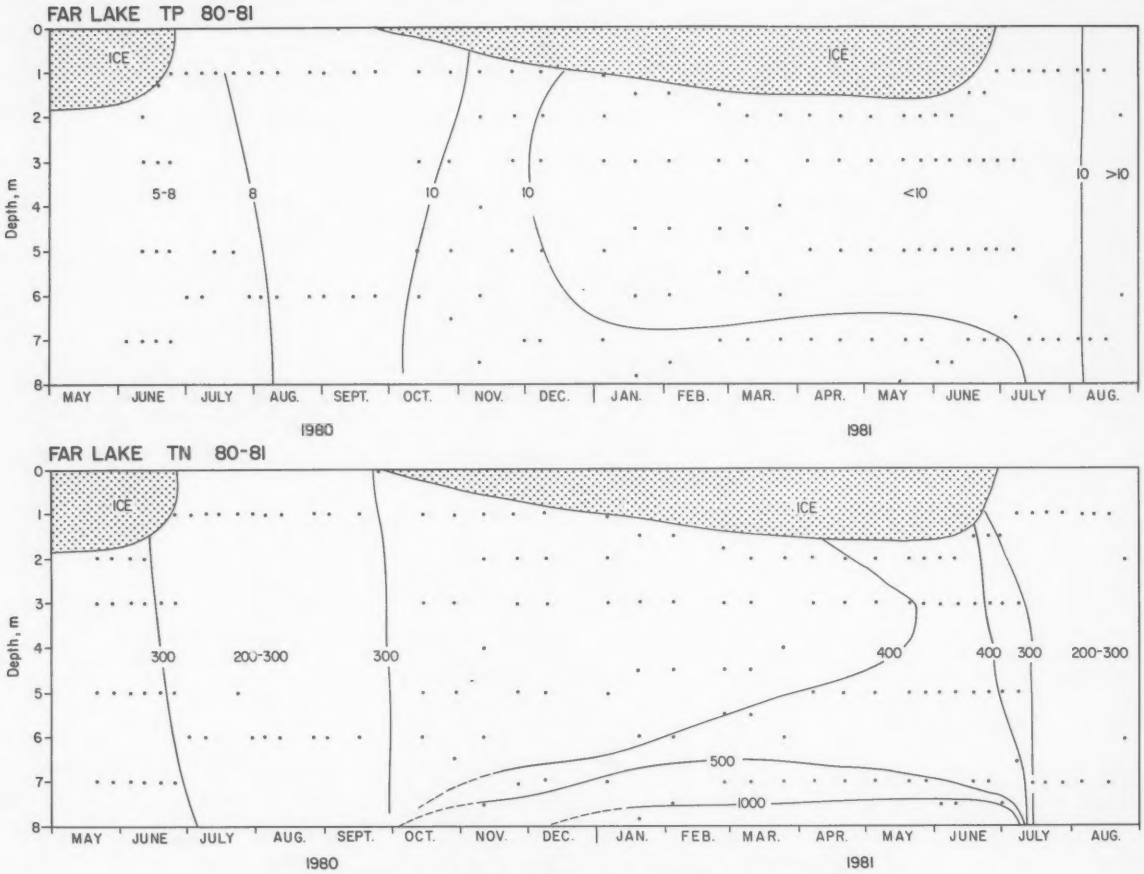


FIG. 4. Concentrations of TP and TN ($\mu g \cdot L^{-1}$) in Far Lake during 1980–81.

tion. Protozoan populations were generally variable, but variability increased even more after eutrophication in P + N Lake (Fig. 2); as an example, 576 $mg \cdot m^{-3}$ on 10 March 1981 dropped to 3 $mg \cdot m^{-3}$ on 10 April and then rapidly rose to the record high of 11 282 $mg \cdot m^{-3}$ on 21 May 1981 (Fig. 2). Protozoan biomass tended to be a variable but small fraction (1–10%) of phytoplankton biomass under oligotrophy whereas the ratio of mean protozoan to mean phytoplankton biomass increased annually from 0.10 to 0.38, 0.56, and 1.44 during 1979–81 in P + N Lake after fertilization.

Nutrients

Total P concentrations did not vary much throughout the year, as typified by Far Lake in 1980–81 (Fig. 4). There was often a spring minimum about the time of ice-out and a brief maximum in early winter, with relatively high levels in the bottom water. Summer open water concentrations were typically 5–10 $\mu g \cdot L^{-1}$ except in P + N Lake after fertilization, when they reached 10–20 $\mu g \cdot L^{-1}$. No pattern was detectable in summer concentrations in any lake, after the minimum at ice-out. Mean summer open water P concentrations probably increased after fertilization in P + N Lake, although an upward trend is also present for 1979–81 in Far and Spring lakes (Fig. 5). There was also a general increase with time in the variance associated with P concentration measurements, which makes none of the changes in annual mean open water P concentrations significant at $P = 0.05$. Retention of P (R_p) averaged about zero in control Spring Lake, and in Jade Lake after fertilization ceased, whereas R_p averaged 90% in P + N, Far, and Jade lakes during H_3PO_4 addition (Fig. 6; Welch and Legault 1986).

N typically reached a brief minimum in late June immediately prior to ice-out (Fig. 4). Summer open water concentrations displayed no patterns in any of the lakes. Concentrations increased throughout the water column over winter, especially in the bottom water, where TN reached 1200 $\mu g \cdot L^{-1}$ in Far Lake (Fig. 4), 1100 $\mu g \cdot L^{-1}$ in Spring Lake (not shown), and 3000 $\mu g \cdot L^{-1}$ in P + N Lake by 1981 (not shown). There appeared to be an increase in TN in P + N Lake as a result of fertilization (Fig. 5), although like P, variance increased through time, obscuring any small trends there might have been.

Silica displayed the strongest seasonal cycles of the three nutrients. Si typically dropped to a minimum of about 100 $\mu g \cdot L^{-1}$ shortly after ice-out (Fig. 7), slowly increased in concentration in August (Welch and Legault 1986, their fig. 13, P + N Lake), and returned from the sediments strongly over winter, reaching bottom water concentrations over 500 $\mu g \cdot L^{-1}$ in Far Lake (Fig. 7), 800 $\mu g \cdot L^{-1}$ in Spring Lake (not shown), 1600 $\mu g \cdot L^{-1}$ in Jade Lake (not shown), and 1000 $\mu g \cdot L^{-1}$ in

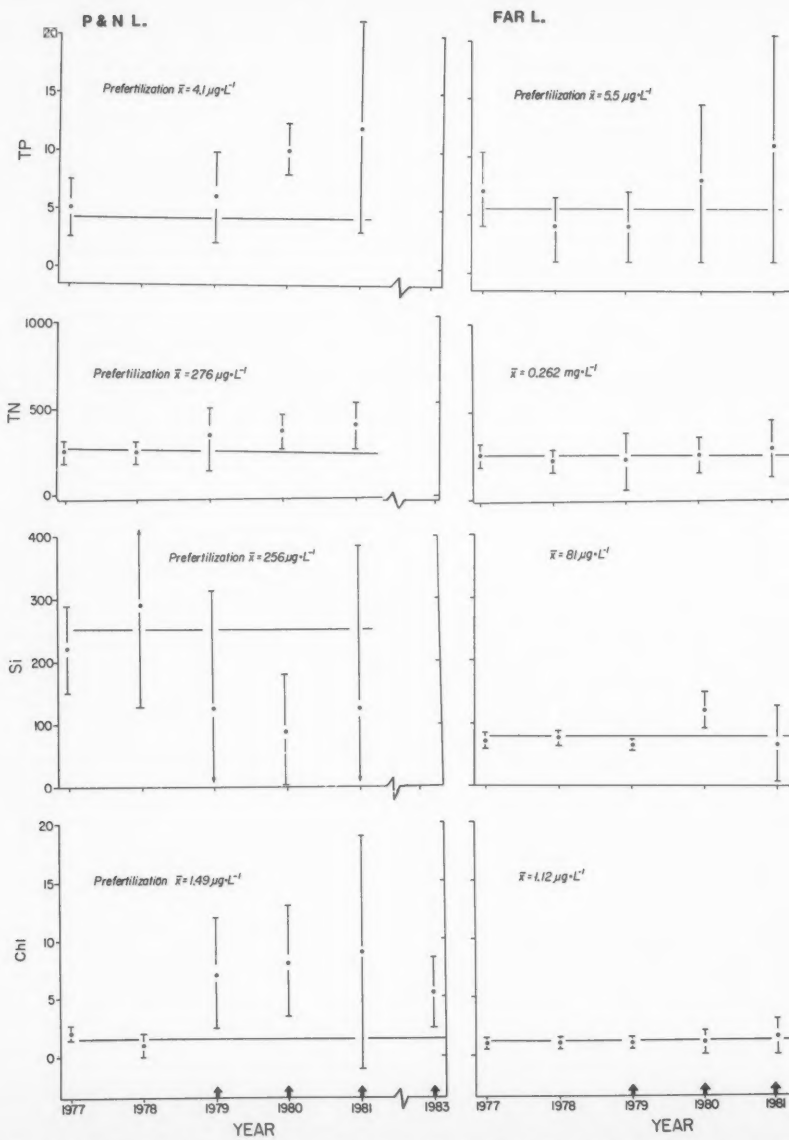
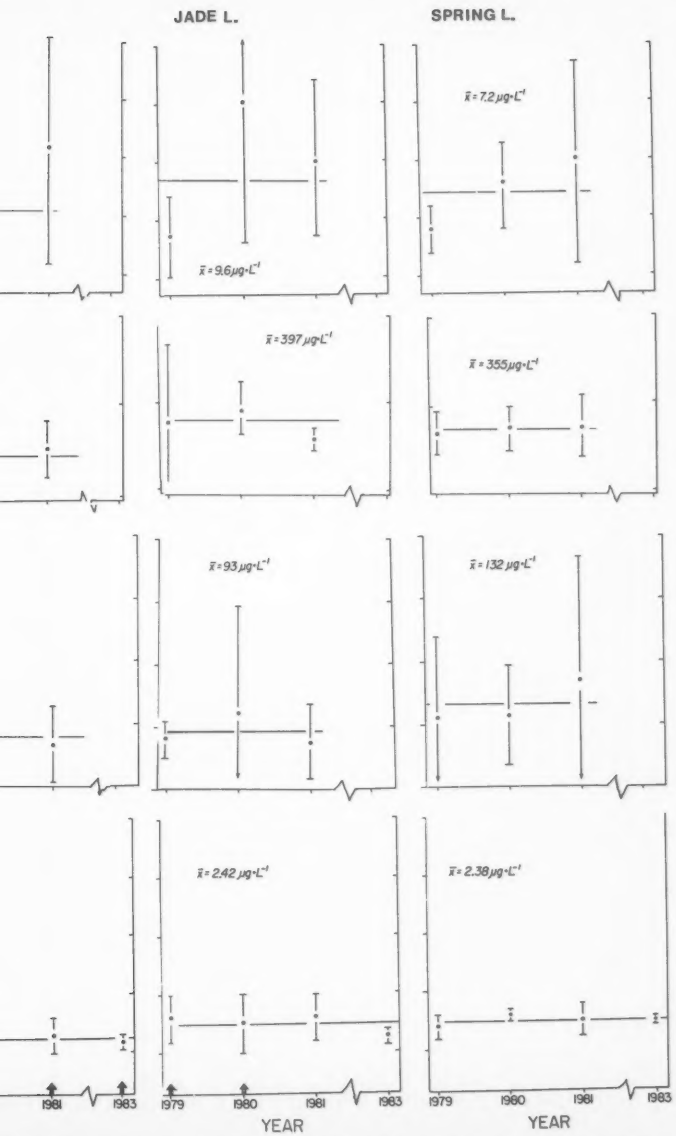


FIG. 5. Mean open water concentrations (horizontal lines) of P, N, Si, and Chl *a* in Saqvaqjuac lak
±2 SD of annual mean. Arrows indicate years when fertilization occurred.



juac lakes. Horizontal lines represent prefertilization mean and vertical lines indicate

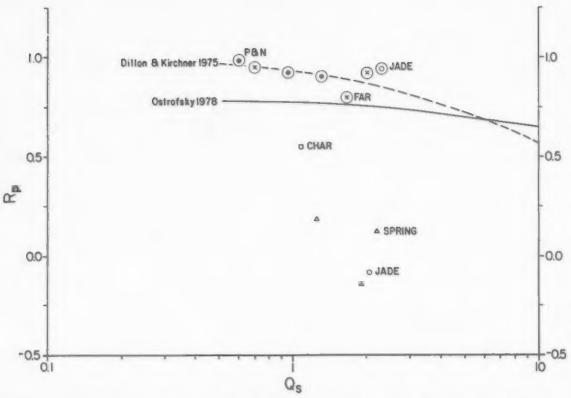


FIG. 6. Retention of P (R_p) as a function of water loading (Q_s, $m^3 \cdot m^{-2}$ lake surface). Δ, Spring Lake, control; ○, Jade Lake after fertilization ceased; ●, P + N Lake during fertilization; χ, Far Lake during fertilization. Solid line is the best fit of the model selected by Ostrofsky (1978) to describe R_p in 53 Yellowknife-area lakes. Broken line (Dillon–Kirchner model) describes southern Ontario lakes.

P + N Lake (Welch and Legault 1986, their fig. 13). Summer open water concentrations of Si appear to have decreased as a result of fertilization in P + N Lake (Fig. 5). Summer Si concentrations never fell below 100 $\mu g \cdot L^{-1}$ before fertilization, whereas during 1979–81, concentrations below 100 $\mu g \cdot L^{-1}$ were common. In Jade Lake, summer Si concentrations fell below 50 $\mu g \cdot L^{-1}$ during fertilization (not shown) but we have no comparative prefertilization data for Jade Lake.

Chlorophyll *a*

Mean open water Chl *a* ranged from 1 to 2.4 $\mu g \cdot L^{-1}$ in unfertilized Saqvaqjuac lakes (e.g. P + N Lake, Fig. 8). After fertilization with P and N, P + N Lake Chl *a* responded immediately and dramatically, increasing about five times, with greatly increased variability during fertilization years (Fig. 5). In Jade Lake the lack of prefertilization data hinders interpretation. When fertilization with P and N ceased in 1981 the Chl *a* did not decrease, but it became lower by 1983 (Fig. 5). Fertilization with P only in Far Lake did not change the mean summer open water Chl *a* but probably did increase the variability of Chl *a* concentrations during the summer (Fig. 5). Thus, Chl *a* levels increased when P and N were added together but did not increase detectably under P addition alone; however, addition of either P only or P and N increased the day to day variability of Chl *a* concentration.

Whole-Lake Photosynthesis

The seasonal progression of C fixation was typically characterized by sharp peaks and dips (Fig. 9 and 10). Peak production did not always occur shortly after ice-out as it usually does in temperate lakes, but could occur anytime, from before ice-out (e.g. P + N Lake, 1978, Fig. 9) to August (P + N Lake, 1981). When primary production was normalized to Chl *a*, P + N Lake postfertilization rates were comparable with prefertilization rates, but for most lake years the peaks were larger (compare especially Far and P + N lakes in 1977–78 in Fig. 9). These peaks and troughs were not due primarily to variable light inputs because the peaks did not all coincide. Although P + N Lake responded immediately to fertilization in 1979 (Fig. 9), it appears to have been increasing its photosynthetic capacity through August 1981 when data collection stopped.

Total annual phytoplankton production about doubled in P + N Lake after fertilization, from ~15 g $C \cdot m^{-2}$ in 1977–78 to ~30 g $C \cdot m^{-2}$ in 1980–81 (Fig. 11), although this was less than the fivefold increase in planktonic Chl *a* after fertilization (Table 2). Far Lake showed no change in phytoplankton photosynthesis before and after P fertilization (Fig. 11). A very high proportion of total photosynthesis occurred during the ice-on period in these lakes, depending greatly upon the snow and slush ice cover during a particular year. Thus in 1977–78 when snow cover was relatively low (Welch et al. 1987), about 60% of photosynthesis occurred during the ice-on period. Chl *a* concentrations just below the ice began to build up in March and reached over 7 $\mu g \cdot L^{-1}$ in the top 3 m in both Far and P + N lakes in early May 1978, decreasing back to 1–2 $\mu g \cdot L^{-1}$ in late

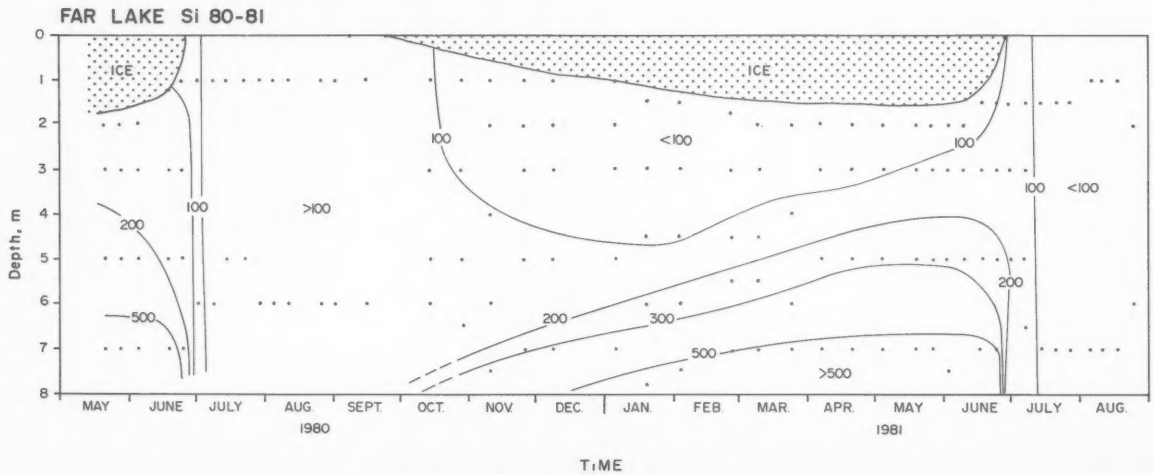


FIG. 7. Concentrations of Si ($\mu g \cdot L^{-1}$) in Far Lake during 1980–81.

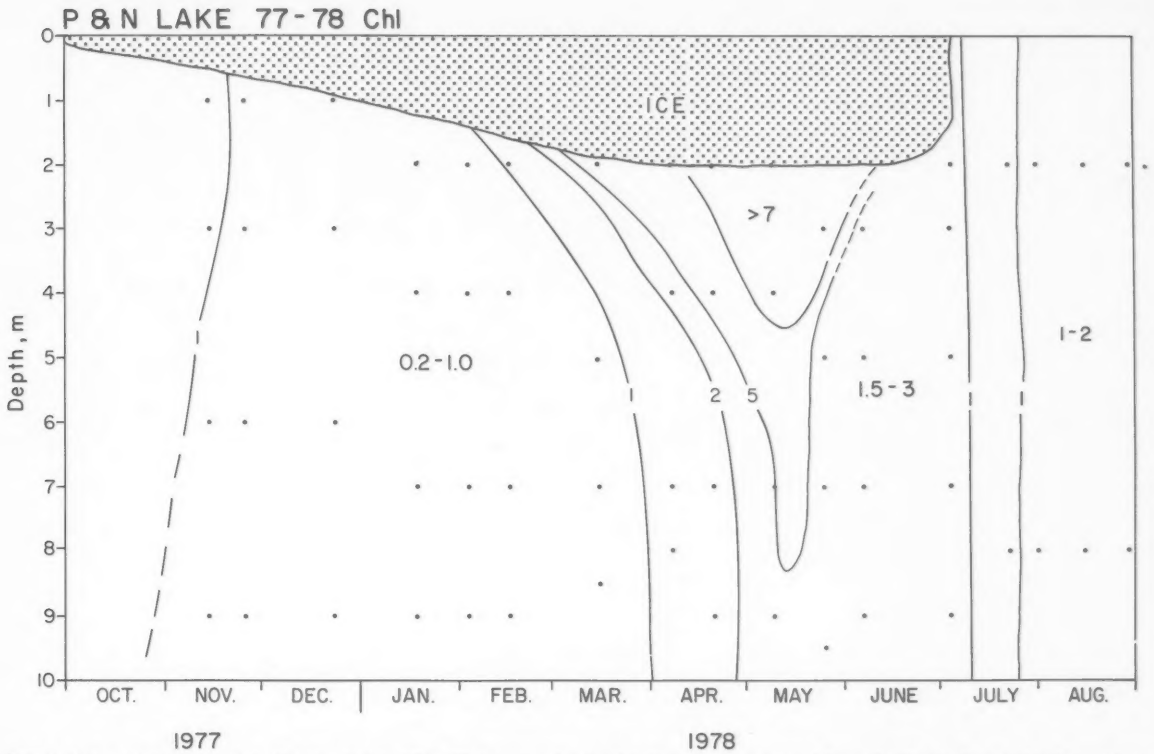


FIG. 8. Chl *a* isopleths for P + N Lake during 1977–78. Summer levels of 1–2 $\mu g \cdot L^{-1}$ are normal for unfertilized oligotrophic Saqvaqjuac lakes, and there is often a bloom of several $\mu g \cdot L^{-1}$ beneath meeting ice in late June. However, the high Chl *a* peak in early May 1978, also found in Far Lake, was probably unusual and resulted from relatively low snow cover through April 1978.

June (Fig. 8). In 1980–81, a similar late-winter increase in phytoplankton did not occur, probably because of lower light penetration (Welch et al. 1987), and under-ice production was only 25–40% of the annual total, depending upon the exact time period selected (Fig. 11). In Jade Lake, less than 10% of annual production occurred beneath the ice (Fig. 11) because a very high proportion (77%) of the lake volume is frozen by spring. Open water photosynthesis in Jade Lake was quite high in 1980 but dropped in 1981 when fertilization stopped (Fig. 11).

Discussion

Phytoplankton in Saqvaqjuac Lakes

Although all the Saqvaqjuac lakes investigated which were well above sea level were typically oligotrophic, some other lakes a few metres above sea level had much higher phytoplankton biomass (Welch and Legault 1986). For example, Green Tea Lake (Welch 1985) at 1.5–7.2 $g \cdot m^{-3}$ would be classified as mesotrophic by Vollenweider's criteria (1968). This lake was permanently green during 1976–83 and was green in topographic survey aerial photos taken in 1956. It was dominated by the cyanophytes *Oscillatoria* spp. and N_2-fixing *Anabaena* spp. Also, Highway Lake (see photo in Welch and Legault 1986), Lake 134, and several other low-elevation lakes on the coast north of Saqvaqjuac Inlet also have permanent cyanophyte blooms. Although one unfertilized reference system, Spring Lake, did not have a permanent population of cyanophytes, it was near sea level and its maximum summer biomass ranged from 1.4 to 6.8 $g \cdot m^{-3}$ during 1979–81, classifying it as mesotrophic. We have suggested elsewhere that excess salts and P entering these lakes after uplift from the sea result in a brief "green" transition period lasting perhaps a few hundred years after freshening (Welch and Legault 1986), and we think that is why Spring Lake has above-average phytoplankton biomass.

Cyanophytes are normally rare in the plankton of arctic lakes, presumably because they are sensitive to photooxidation at low water temperatures (Abeliovich and Shilo 1972). Although cyanophytes did not increase in the plankton of Far Lake after P-only fertilization, the percentage of N_2-fixing species did, and the percentage of N_2 fixers increased in benthic epilithiphyton as well (H. J. Kling, unpubl. data). Holmgren (1983) also found that N_2 fixers increased in the benthos of P-fertilized Lake Hymenjaure and suggested that the lower light and more stable conditions on the lake bottom allowed them to avoid photooxidation. But the blooms in Green Tea and other Saqvaqjuac lakes show that planktonic cyanophytes can do well in the arctic. Schindler et al. (1974b) also found *Anabaena* to be a dominant summer phytoplankter in Meretta Lake, Resolute (75°N). Photooxidation cannot therefore be the only factor limiting cyanophytes at high latitudes; low nutrient concentrations appear to be at least as important.

Chlorophyta typically have high diversity and low biomass in high-latitude lakes (Prescott 1963; Holmgren 1983) and respond positively to increased nutrients, although it may be several years before a stable assemblage occurs. Thus, P + N

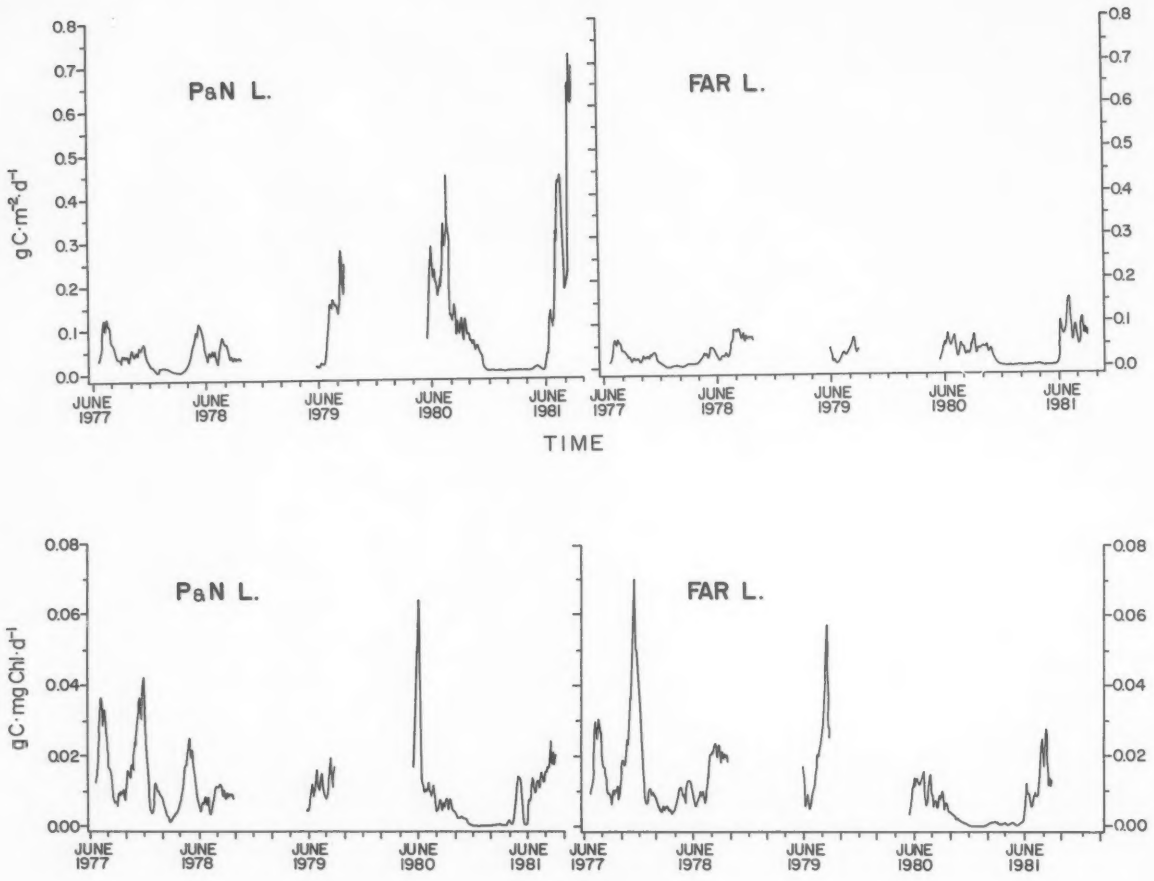


FIG. 9. Phytoplankton production in P + N and Far lakes.

Lake experienced only slight increases in Chlorophyta in the first 2 yr of fertilization but sustained a massive bloom of the volvocalean *Paulschulzia pseudovolvox* in the third year (Table 4; Fig. 2). In the Swedish Kuokkel area, all the P- and N-fertilized lakes developed monocultures of chlorococcal greens after a few years (Holmgren 1983, 1984). A Norwegian study (Reinertsen 1982) showed an increase in chlorococcal greens plus desmids during the second year of N and P additions, corresponding to the highest level of PO_4-P (12 $\mu g \cdot L^{-1}$) recorded from the epilimnion of Lake Langvatn during the 1974–78 period (Reinertsen 1982). The first 3 yr of P and N fertilization in Lake 227 (ELA) saw an increase in desmids, but by the fourth year of eutrophication, chlorococcal species dominated (Findlay and Kling 1975). Certain species of Volvocales have low temperature tolerances as well as high light requirements and are thus often found directly under the ice, e.g. in polluted Meretta Lake (75°N; Kalff et al. 1975); as mobile flagellates they are better adapted to respond to increased nutrients under ice than chlorococcal greens.

Chrysophyceae generally dominate the phytoplankton of nutrient-poor arctic and subarctic lakes (Kalff et al. 1975; Moore 1981; Holmgren 1983; Kalff and Watson 1986; this study), as they do in naturally oligotrophic lakes in temperate areas of the Canadian Shield (Kling and Holmgren 1972; Findlay and Kling 1975; Janus and Duthie 1979). Chrysophytes respond initially to eutrophication but eventually are taken over by Chlorophyceae, sooner at midlatitudes, later in the arctic. Although Kalff and Watson (1986) have shown that chrysophytes are less common in tropical than in high-latitude lakes, those authors attributed the differences to differing trophic states rather than latitudinal effect per se, and they have presented evidence (e.g. commonality of phytoplankton species from pole to equator) that phytoplankton species abundance is controlled more by physical factors such as stratification and nutrient status than by temperature and snow cover. Although patterns of phytoplankton succession are beginning to emerge, predictability across a wide variety of lake types remains poor.

Protozoa

Protozoan energetics in lakes have received little attention relative to their probable importance in lake metabolism. Rigler et al. (1974) found that ciliate production was 10 times greater than annual rotifer production but only one eighth that of the copepod *Limnocalanus macrurus* in Char Lake, where mean protozoan biomass was 51 $mg \cdot m^{-3}$, comparable with that in

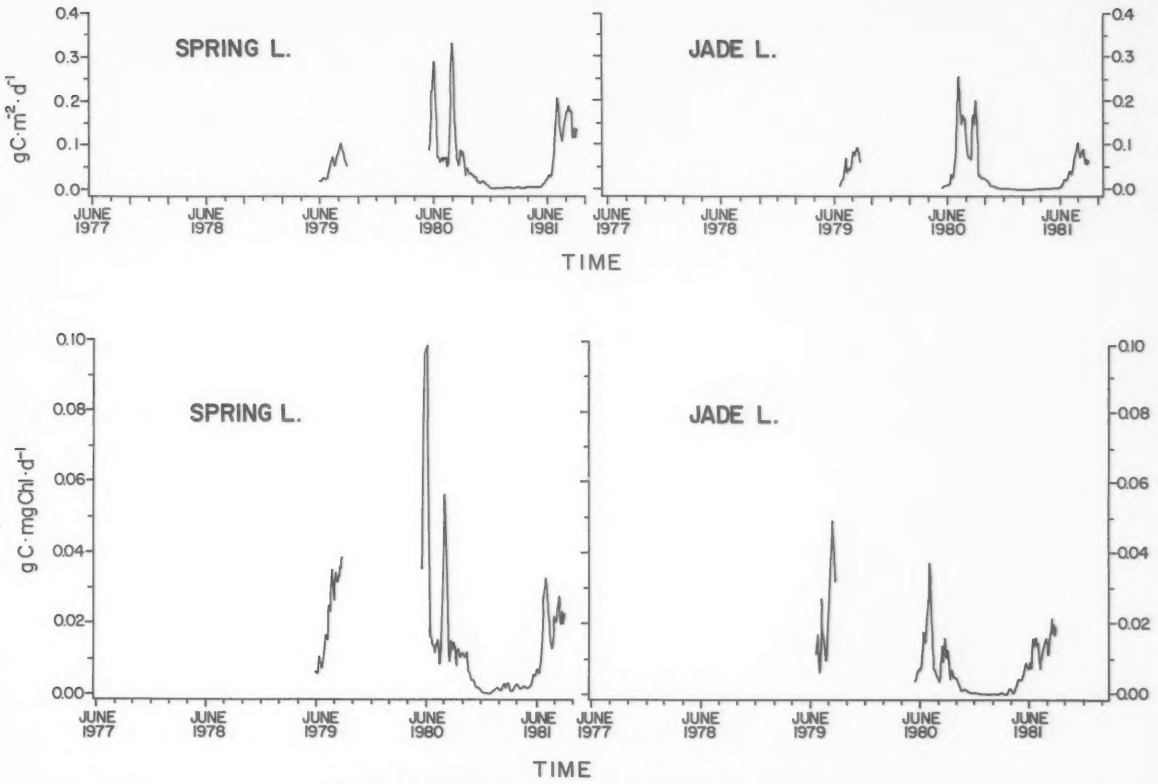


FIG. 10. Phytoplankton production in Spring and Jade lakes.

oligotrophic Saqvaqjuac lakes. Protozoan abundance is roughly proportional to lake trophic status, with peaks often occurring during or shortly after algal blooms (Nauwerck 1963; Wilbert 1969; H. J. Kling, unpubl. data from hypertrophic prairie lakes). Protozoa biomass in P + N Lake after nutrient addition was extremely variable (Fig. 2), for what reasons we do not know. Protozoa are as important in arctic lakes as they are in the temperate zone, but much more research is required if their role is to be understood.

Nutrients

The retention of P (R_p) by P + N Lake averaged 93% during fertilization whereas R_p for Spring Lake, and Jade Lake after fertilization stopped, was near zero (Fig. 6). R_p for fertilized Saqvaqjuac lakes was closely predicted by the Kirchner–Dillon P retention model (Kirchner and Dillon 1975; Dillon and Kirchner 1975) whereas R_p for unfertilized (= low P supply) lakes was nowhere near that predicted (Fig. 6). Ostrofsky (1978) found that the original Kirchner–Dillon model also predicted erroneously high P concentrations in Yellowknife lakes because it overestimated R_p at low water loading. Using the original Kirchner–Dillon data set, Ostrofsky derived a number of statistically indistinguishable models which did not require R_p = 1.0 at zero water supply, as did the original model. When Ostrofsky used his revised model of R_p to predict P concentrations in Yellowknife lakes (~62°N), the observed P concentration was still in excess of predicted P concentration in the water loading range of 0.5–20 $m \cdot y^{-1}$, suggesting that his revised model still overestimated R_p, but not as badly as the Kirchner–Dillon model did (Fig. 6).

What seems to be happening is that arctic lakes which experience very low P supply also have very low R_p whereas under very high (artificial) P supply, R_p becomes very high (Fig. 6). This is partly because the natural P input in arctic lakes is highest in the spring while the lakes are still ice-covered and inflowing water tends to flow beneath the ice and leave the lakes without mixing throughout the water column (Bergmann and Welch 1985; Welch and Legault 1986). P retention models based upon temperate lakes, where P supply is relatively high and occurs during the ice-free season, will therefore overestimate R_p in oligotrophic arctic lakes.

N concentrations increased in P + N Lake after fertilization (Fig. 5) although, like P, the increase was only a small fraction of the total added, indicating rapid uptake and sedimentation. Changes in N concentrations were not detectable in the other lakes. When subarctic and temperate lakes are enriched with P, they gradually enrich their N stocks by N_2 fixation, either planktonic or benthic (Schindler 1977; Lundgren 1978; Persson et al. 1975; Flett et al. 1980). Far Lake did show enhanced N_2 fixation by epibenthic algae (M. Bergmann, Freshwater Institute, pers. comm.), but no change in total N was detectable after 3 yr of fertilization with P only (Fig. 5). Increased variance (Fig. 5) suggests that P addition was having some effect upon N, and a long-term addition of P only might eventually result in increased stocks of N in Saqvaqjuac lakes. Schindler et al. (1987) reported that even after 18 yr of fertilization, the

TABLE 6. Phytoplankton C fixation and nutrient supply ratios at Saqvaqjuac and ELA[1] (calculated from Fee 1979, his table 1).

Lake	Lake years	Mean depth (m)	Annual C fixed ($g \cdot m^{-2} \cdot yr^{-1}$)	C:N:P	Treatment
114	2	1.7	21.7	621:26:1	None
120	1	7.6	14.9	339:25:1	None
223	2	7.2	22.2	593:29:1	None
224	2	11.6	16.6	572:27:1	None
239	4	10.5	16.6	344:25:1	None
240	2	6.1	15.3	232:35:1	None
302S	4	5.1	30.8	994:26:1	None
303	1	1.5	30.6	785:17:1	None
305	1	15.1	9.2	533:22:1	None
			$\bar{x}$ = 20.9	$\bar{x}$ = 557:26:1	
				$\bar{x}$ C:N = 21:1	
226N	4	5.7	67.9	182:7:1	N and P added
227	4	4.4	107.4	209:10:1	N and P added
303	2	1.5	52.9	230:17:1	N and P added
			$\bar{x}$ = 76.1	$\bar{s}$ = 207:11:1	N and P added
				$\bar{x}$ C:N = 19:1	
P + N	1978	3.3	4.7	955:34:1	None
Far	1978	3.6	8.9	318:21:1	None
Spring	1980–81	2.7	15.6	1076:56:1	None
Jade	1981	1.8	7.4	394:42:1	Postfertilization
			$\bar{x}$ = 11.6	$\bar{x}$ = 686:38:1	
				$\bar{x}$ C:N = 18:1	
P + N	1980–81	3.3	31.0	276:15:1	N and P added
Far	1980–81	3.6	9.2	78:6:1	P added
Jade	1980	1.8	12.2	57:15:1	N and P added

N budget in Lake 227 (ELA) had not reached a new steady state, although P had equilibrated after a decade.

Si was retained much more efficiently ($\bar{x}$ = 75%) in Saqvaqjuac lakes than was naturally supplied P ($\bar{x}$ = 2%) because Si loading was highest in late summer when the lakes were mixed and biological demand was high whereas P input was highest in spring. Since diatoms were not a major component of the phytoplankton but did dominate the epibenthos, the rapid Si reduction in early summer was probably due mostly to benthic algal uptake. The typical open water concentrations of ~100 $\mu g \cdot L^{-1}$ were far below the 400 $\mu g \cdot L^{-1}$ level at which diatom growth becomes limited (Parker et al. 1977; Tilman et al. 1982), so it is not surprising that spring phytoplankton blooms in Saqvaqjuac lakes were nondiatom.

After fertilization with P and N, summer Si levels fell as low as 50 $\mu g \cdot L^{-1}$ (unpubl. data). In polluted Meretta Lake (75°N) at Resolute, N.W.T., excess P and N loading from the discharge of domestic sewage resulted in summer Si levels below 20 $\mu g \cdot L^{-1}$ (Schindler et al. 1974b). The Saqvaqjuac and Meretta results support the contention of Schelske et al. (1986) that recent severe Si depletion in the lower Great Lakes has resulted from increased P loading and subsequent diatom growth, so that diatoms are now Si limited.

In the fall of 1980, diatom blooms occurred in P + N, Far, and Spring lakes. This was probably in response to the high silica loading that occurred during the unusually wet summer and autumn of 1980, and we doubt that such a diatom bloom would occur in the fall of a year such as 1981, which had a very dry summer with low Si input (Welch and Legault 1986, their fig. 6 and 7).

Photosynthetic Response to Light and Nutrients

The ratio of phytoplankton C fixed per unit P supplied was slightly higher for unfertilized lakes at Saqvaqjuac compared with ELA, 38:1 versus 26:1 (Table 6); C:N ratios were lower, 18:1 versus 23:1 at ELA (Table 6), and the ratio of C fixed per unit light entering the water was slightly lower at Saqvaqjuac, 4.37 mg $C \cdot E^{-1}$ versus 4.60 mg $C \cdot E^{-1}$ at ELA (Table 6). Although the differences in these three ratios are not statistically significant, they do suggest that Saqvaqjuac phytoplankton are even more P limited than those at ELA. After surface fertilization during the open water season with P and N, C:P ratios dropped sharply at both Saqvaqjuac and ELA, as expected in P-limited systems where added P is less efficiently cycled, whereas C:N ratios did not change much (Table 6). Fertilized ELA lakes developed much higher chlorophyll concentrations and higher C:light ratios presumably because their fertilization rates were much higher, e.g. 0.5 g $P \cdot m^{-2} \cdot yr^{-1}$ in Lake 227 compared with 0.1 g P in P + N Lake.

Photosynthetic Parameters

The use of only four (paired) points on the *P/I* curve (Fig. 1) resulted in generally low precision for α and P_m^B, but the large number of data sets (N = 210 for all lake years) allowed comparisons to be made. The relative frequency distributions of "summer" α and P_m^B were not detectably different among Far, Jade, and P + N pre- and postfertilization data (not shown) whereas Spring Lake tended toward higher α (Fig. 12) and P_m^B (Fig. 13) when compared with the other lakes. We compared photosynthetic parameters for "summer" and "winter," empirically defining "summer" as 15 June – 30 September because sharp changes in subsurface light, α, P_m^B, and I_k occurred at those times. Winter P_m^B values were slightly lower than in summer (Fig. 13). Winter α values were much higher than in summer, peaking at 11 (Fig. 12) compared with the summer peak of 3.5. The greater difference in mean α between

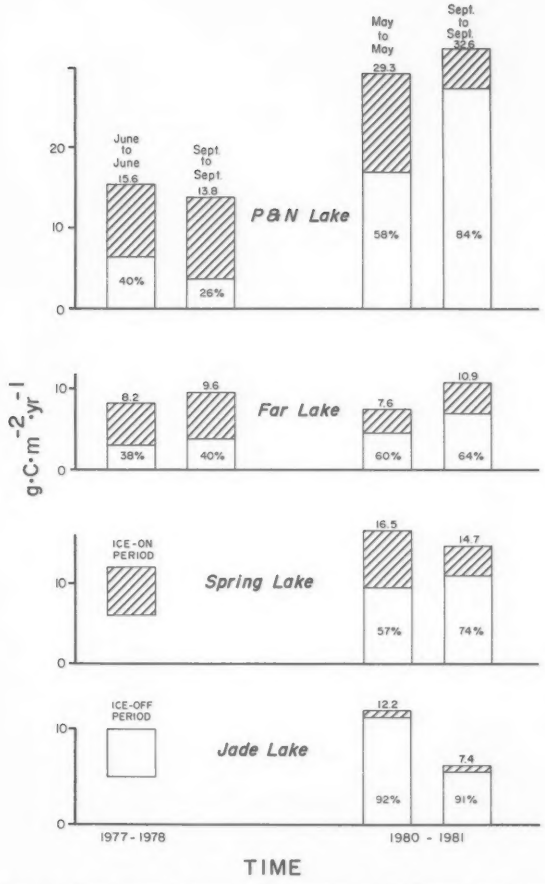


FIG. 11. Total annual phytoplankton production in Saqvaqjuac lakes.

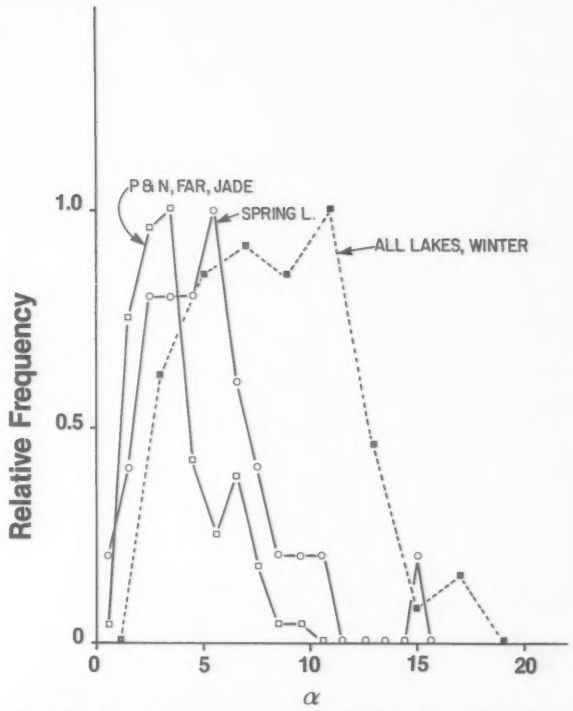


FIG. 12. Frequency distributions of α. The distributions have been normalized by dividing them by the modal frequency. P + N, Jade, Far lakes, summer, all years (N = 100); Spring Lake, summer (N = 29); winter in all lake years (N = 81). Summer is defined here as 15 June – 30 September. We have not shown 17 winter values of $\alpha > 20$ because they were probably artifacts caused by the application of the hyperbolic tangent function to P/I curves with very low ^{14}C uptake.

"summer" and "winter" was probably not directly due to temperature because many of the higher "summer" values of α occurred beneath the ice in June and early July when lake temperatures were only 2–4°C.

α and P_m^B interact to define I_k, the light intensity at the onset of light saturation (Fig. 1). I_k averaged about 15 $\mu E \cdot m^{-2} \cdot s^{-1}$ in winter but increased sharply in June, peaked around 140 in July, and dropped back abruptly in September (Fig. 14). Comparison with the mean light entering the water column ("0 m" in Fig. 14) suggests that I_k lagged behind light about 2–3 wk. From June to September, Saqvaqjuac lakes were essentially isothermal and freely circulating, with only temporary thermal stratification (Welch et al. 1987), and phytoplankton should have been almost continually exposed to the entire light range in the water column, so 2–3 wk may be the average physiological response time of the phytoplankton. Coté and Platt (1983) also found that I_k followed light in well-mixed Bedford Basin, with peak I_k occurring about 10 d after the June solstice (their fig. 20).

Chlorophyll *a*

Because the open water season is so short in the arctic, we have used mean open water TP rather than mean spring TP as

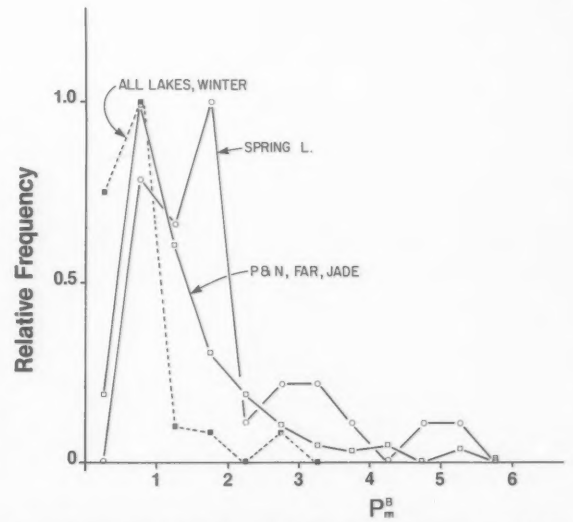


FIG. 13. Frequency distributions of P_m^B. The distributions have been normalized by dividing them by the modal frequency. P + N, Jade, Far lakes, summer, all years (N = 100); Spring Lake, summer (N = 29); winter in all lake years (N = 81).

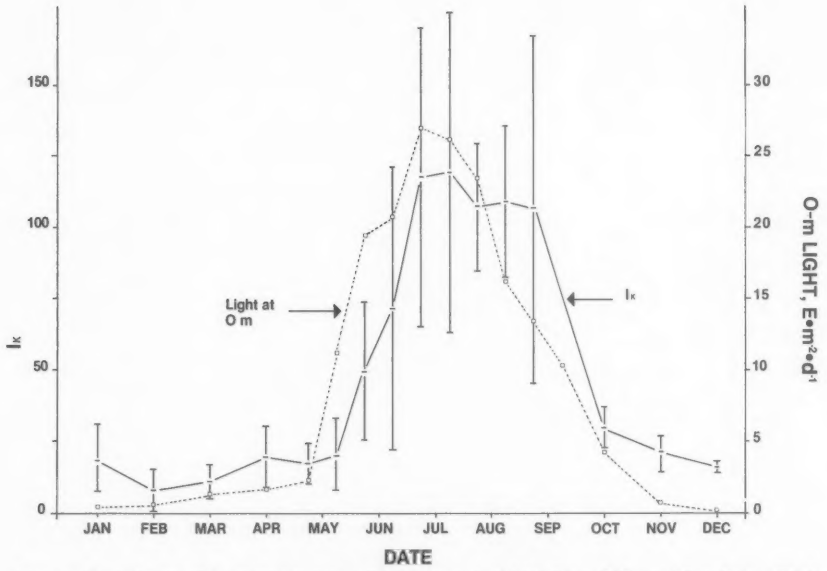


FIG. 14. Distribution of I_k during the year, all lake years combined (N = 210), and 0-m light, all lake years combined. Zero-metre light is calculated as light penetrating the unfrozen water surface.

a predictor of mean summer Chl *a*. With the exception of P + N Lake during fertilization, Chl *a* in Saqvaqjuac lakes was predicted well by Smith's (1982) relationship between Chl *a* and TP for 127 North American lakes, and the variability at a given mean TP concentration, two to three times, is similar to that seen for comparable data sets (ELA, South Indian Lake (SIL), Yukon) (Fig. 15). Saqvaqjuac lakes (except P + N during fertilization) form a continuous extension of the ELA and SIL data sets, although there is little overlap of the SIL data with ELA (5 of 16 points in Fig. 15), probably because SIL is more light limited due to suspended sediment than either Saqvaqjuac or ELA (Fee et al. 1987). Since Saqvaqjuac lakes average colder than the lakes used to derive the relationship, we conclude that temperature per se does not affect the steady-state biomass response of phytoplankton to nutrient levels.

There are few arctic data to compare with Saqvaqjuac Chl *a* : TP Oligotrophic Char Lake (75°N) falls well below the line in Fig. 15 whereas Chl *a* in nearby Meretta Lake responded to large additions of sewage as predicted, despite a low N:P ratio of about 13. Two lakes on the Colville delta, Alaska (65°N), had very low Chl *a* : TP, and lake Stugsjon (68°N) in subarctic Sweden was also low. The shallowness of these three lakes (mean depth 1.2 m for Stugsjon, 1.2 for Colville Lake E, 1.6 m for Colville Lake C) may have had an effect on the Chl *a* : TP relationship. In both Lake Stugsjon (Jansson 1979) and Char Lake (Welch and Kalff 1974), 80% of total photosynthesis occurs on the bottom, and the Colville lakes are probably similar; this may have reduced the ability of the phytoplankton to respond to P.

We have no explanation for the somewhat higher than expected response of mean summer Chl *a* and phytoplankton biomass to fertilization in P + N Lake (Fig. 15). At high latitudes, phytoplankton may utilize high levels of nutrients more efficiently that at lower latitudes simply because light input during the summer is higher and more continuous. It may be significant in this regard that P + N Lake, Meretta Lake, and to a lesser extent the ELA lakes all show higher than expected responses of mean summer Chl *a* to added P compared with low-latitude lakes (the line in Fig. 15). Also, Smith (1982) found that Florida lakes had lower than expected Chl *a* per unit P and N. The Florida data set used annual averages on the grounds that the lakes were ice-free and active year-round. Thus what may be occurring is that with increasing latitude the time window (= summer open water season) for data selection is compressed, resulting in higher Chl *a* : TP for shorter periods of time. Certainly if Chl *a* : nutrient data were placed on a mean annual basis, rather than a mean summer open water basis, there would be a strong positive correlation between deviation from Chl *a* : P models and increasing latitude. The higher than expected response by P + N Lake may therefore simply be an artifact of sampling.

Response to Fertilization

In general, Saqvaqjuac lakes responded to fertilization much as have other experimental lakes. When P and N were added together, response by phytoplankton was immediate and sustained (Fig. 2 and 5), as has been the case at ELA (Schindler 1977; Schindler et al. 1978; Fee 1979), lakes in the Kuokkel area (Holmgren 1984; Persson et al. 1975; Jansson 1979), British Columbia coastal lakes (Stockner and Shortreed 1985), a Colville delta lake (McCoy 1983), and Meretta Lake in the high arctic (Kalff et al. 1975). P alone did not increase productivity at Saqvaqjuac (Fig. 2 and 5), despite generally high N:P ratios (Table 6). Likewise the response of Chl *a* and photosynthesis to P-only fertilization of Lake 261 at ELA was within the 95% confidence limit in 2 yr and only slightly above the limit in the other 2 yr (Fee 1979). P-only fertilization of a shallow Colville delta lake gave a 10-fold increase in phytoplankton Chl *a*, although this was dwarfed by another 10-fold increase when N was then added (McCoy 1983). Subarctic lakes at Schefferville (Smith et al. 1984) and Lake Hymenjaure in subarctic Sweden

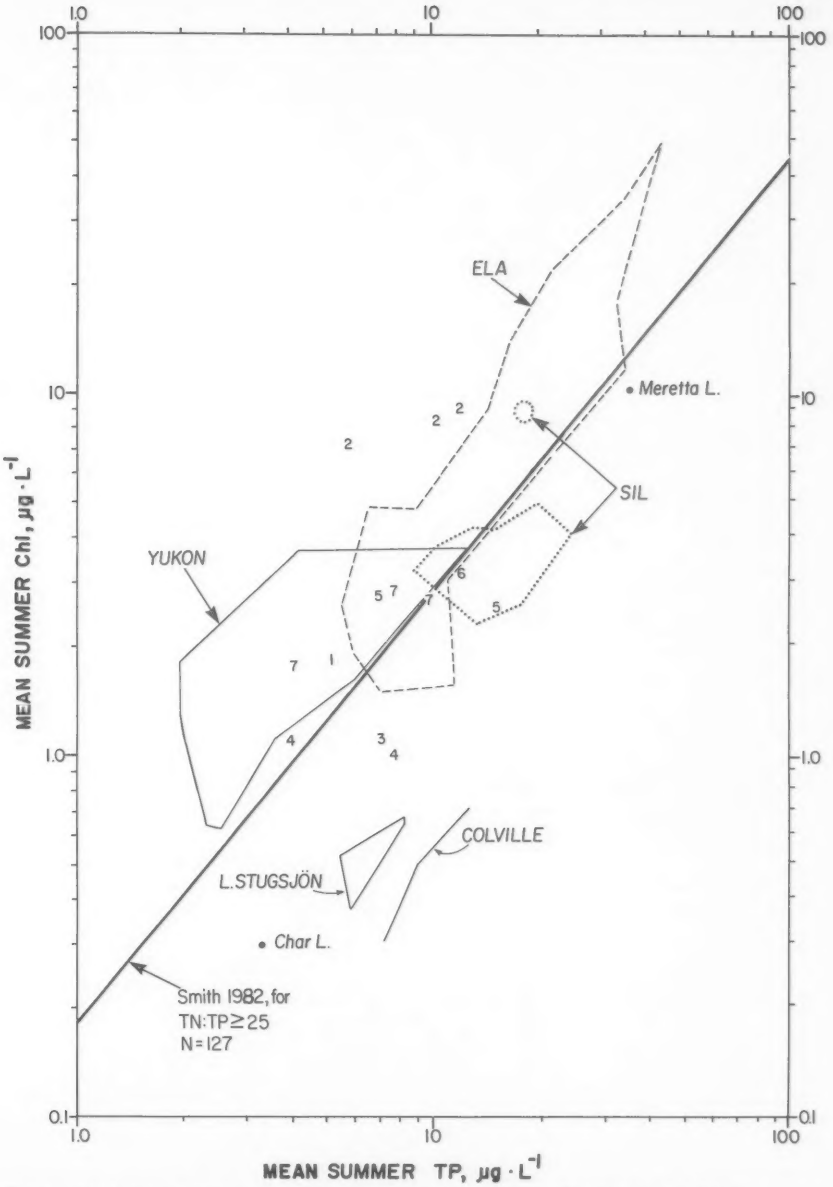


FIG. 15. Relationship between mean summer TP and mean summer Chl *a* for North American lakes.
Line is log Chl a = 0.653 log TP + 0.548 log TN − 1.517 calculated by Smith (1982) for 127 north-temperate lakes assuming TN:TP ≥ 25. It is practically identical to the relationship calculated by Hoyer and Jones (1983) for 157 midwestern reservoirs, where log Chl a = 1.20 log TP − 0.72. Yukon lakes (N = 19) from Shortreed and Stockner (1986); South Indian Lake (SIL) includes seven areas in the main lake and one from nearby Wood Lake, time-weighted means for 5 July – 4 September 1975 (preimpoundment) and mean of 1977–78 (postimpoundment), 16 data points total (R. Hecky, Freshwater Institute, pers. comm.). Experimental Lakes Area (ELA) includes 59 lake years when N:P ≥ 25, excluding experimentally acidified lakes but including those with nutrients added (Lakes 222, 226NE, 227, 302, 303, 304, and 382) (D. Schindler, Freshwater Institute, pers. comm.). Lake Stugsjön (68°27′N) in northern Sweden for years 1971–75 from Jansson (1979); Colville is three lake years on the Colville delta (65°5′N) from McCoy (1983), Meretta Lake TP from Schindler et al. (1974b); Char Lake TP taken as outflow values from Schindler et al. (1974a); Chl a for both from Kalff and Welch (1974) (Meretta N:P ≈13); Saqvaqjuac lakes, 1 = P + N Lake prefertilization, 2 = P + N Lake fertilized, 3 = Far Lake prefertilization, 4 = Far Lake fertilized, 5 = Jade Lake fertilized, 6 = Jade Lake postfertilization, 7 = Spring Lake (control). Additional data sets not shown include coastal British Columbia lakes (Stockner and Shortreed 1985), Alberta prairie lakes (Prepas and Trew 1983), Nova Scotia lakes (Kerekes 1975), and Lake Washington (Edmondson and Lehman 1981).

(Persson et al. 1975) responded to P-only addition with either no detectable change or a slight increase in phytoplankton. All the lakes mentioned above gave evidence of being P limited, but only small amounts of added P resulted in N deficiency. From a practical standpoint there is no doubt that, although oligotrophic lakes north of 50° latitude are primarily P limited, both P and N must be added if any considerable increase in phytoplankton production is desired. Conversely, reduction of phytoplankton would be easiest by controlling P input, as at lower latitudes (e.g. Schindler 1977).

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Slower Growth Results in Larger Otoliths: An Experimental Test with Guppies (*Poecilia reticulata*)

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Reznick, D., E. Lindbeck, and H. Bryga. 1989. Slower growth results in larger otoliths: an experimental test with guppies (*Poecilia reticulata*). Can. J. Fish. Aquat. Sci. 46: 108–112.

We demonstrate that slowly growing guppies (*Poecilia reticulata*) have larger otoliths than equal-sized, rapidly growing guppies. This relationship has been suggested by previous authors, but they compared wild or pond-reared fish from different poopulations, thereby confounding their observations with the different environments. Our experiment controlled for the genetic background, food quality, and the aquatic environment and assessed the influences of food availability and growth rate on relative otolith size. This difference in relative otolith dimensions could aid in comparing growth rates among populations or could improve the use of otolith dimensions for estimating population age structures. In both cases, these methods could also complement data based on counting daily increments or annuli on otoliths and other structures to characterize growth rates and age structures.

Les auteurs montrent que les guppys (*Poecilia reticulata*) à croissance lente présentent de plus gros otolithes que les guppys à croissance rapide de même taille. L'hypothèse d'une telle relation avait déjà été faite par d'autres auteurs, mais ceux-ci avaient comparé des poissons sauvages ou élevés en étangs de populations différentes de sorte que leurs observations se trouvaient obscurcies par les écarts entre les environnements. Dans la présente expérience, les paramètres relatifs aux antécédents génétiques, à la qualité des aliments et à l'environnement aquatique ont été contrôlés et on a évalué les effets de la disponibilité de la nourriture et du taux de croissance sur la taille relative des otolithes. Cet écart de taille relative pourrait permettre de comparer les taux de croissance de populations différentes ou de faciliter l'utilisation de ces paramètres pour l'estimation de la structure des âges des populations. Dans les deux cas, ces méthodes permettraient de compléter les données reposant sur le dénombrement des accroissements quotidiens ou des anneaux des otolithes, ou d'autres structures, et servant à caractériser les taux de croissance et la structure des âges.

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Fish otoliths are valued primarily as an indicator of age; however, they are also valuable for the information they contain on a fish's past history. Such information includes somatic growth rate (e.g. Volk et al. 1984; Radtke et al. 1985; Secor and Dean 1986; reviewed by Campana and Neilson 1985), temperature regime (reviewed by Radtke 1984), periods of low resource availability (e.g. Rice et al. 1985; Jones and Brothers 1987), or transitions between life history stages (e.g. Brothers and McFarland 1981; Victor 1982, 1983; Neilson et al. 1985a).

One way for otoliths to yield information on somatic growth rate is that slowly growing fish appear to have larger and/or heavier otoliths than equal-sized, rapidly growing fish (Templemen and Squires 1956; Wilson 1984; Secor and Dean 1986). If this observation is generally true, then the *relative* size of otoliths could provide an index of relative growth rate. Relative growth rates could therefore be evaluated with statistical comparisons among body size – otolith size regression lines. Otolith size and body size data are far more easily obtained than generating growth curves from counts of daily or annual rings (Secor and Dean 1986). Because these studies were based on either comparisons among different populations or on variations among wild-caught individuals, the ultimate cause of the differences in relative otolith dimensions may be attributable to factors other than growth rate differences, such as unknown environmental effects.

We have experimentally evaluated the relationshp between growth rate and otolith size to see if slowly growing fish have relatively large otoliths. We manipulated growth rate by controlling food availability. It was thus possible to generate similar-sized fish of different ages free from confounding environmental influences.

Materials and Methods

We performed a two-way experiment with high and low levels of food availability, sampling fish after either 6 or 16 wk of controlled feeding. There were therefore four treatments: low food, 6 wk (Lo-6); low food, 16 wk (Lo-16); high food, 6 wk

TABLE 1. Summary of food availability for each 2-wk interval. Hi = high food availability; Lo = low food availability. The first number of each pair refers to the number of microlitres of liver paste fed in the morning; the second number refers to the number of microlitres of shrimp nauplii fed in the afternoon.

Weeks	Hi	Lo
1–2	4, 4	0.5, 0
3–4	7.5, 7.5	0.5, 0.5
5–6	12.5, 12.5	1.0, 1.0
7–8	17.5, 17.5	2.0, 1.0
9–10	25, 20	2.5, 1.5
11–12	30, 30	4.0, 3.0
13–14	40, 40	6.0, 6.0
15–16	50, 50	8.0, 8.0

(Hi-6); and high food, 16 wk (Hi-16). The times of preservation were chosen so that the Lo-16 fish were approximately equal in size to the Hi-6 fish. These two groups of equal-sized fish enabled us to test the hypothesis that slowly growing fish (Lo-16) have relatively large otoliths. The study included the other two groups (Lo-6 and Hi-16) to provide a complete description of growth for each level of food availability through the full term of the experiment and hence to demonstrate that there were no confounding influences between the sixth and sixteenth weeks of the study. The dependent variables included standard length and wet weight, measured at 2-wk intervals, plus the length of the major axis of the sagitta (saccular otolith).

The fish were the third- or fourth-generation descendants of 24 female and 24 male guppies (*Poecilia reticulata*) collected from the upper Aripo River of the Northern Range of Trinidad in February 1984. The mothers of the fish in the experiment were randomly selected from a large outbred stock and isolated in 8-L aquaria until they gave birth. Ten young were saved from each litter and reared in either 19-L aquaria or in bisected 38-L aquaria. Each litter was reared separately so that "litters" could be retained in the data analysis. They were sexed while immature (approximately 13–14 mm standard length), based on the metamorphosis of the anal fin (as described by Turner 1941 for *Gambusia affinis*) or on the presence or absence of melanophores at the base of the abdomen; males do not accumulate such melanophores whereas females do.

We selected four females from each litter and included 20 litters (80 fish) in the experiment. Males were excluded because their growth is determinate, virtually ceasing at maturity, while females have a typically indeterminate growth pattern (D. Reznick, pers. obs.; von Bertalanffy 1938). One female from each litter was randomly assigned to each treatment. Females were housed individually in 8-L aquaria throughout the experiment. The four females from each litter were grouped together, making litters equivalent to randomized blocks in the experimental design. Females were never mated and none reproduced during the course of the study. The age of the litters at the beginning of the experiment ranged from 17 to 44 d, with a mean of 29 d. The date when each litter entered the experiment ranged from 15 May to 25 June 1985, with the variation resulting from differences in the date of birth and the age at the beginning of the study. The results are reported as the number of weeks on controlled feeding, rather than actual age.

The quantified feeding procedures followed the methods of Reznick (1983). Briefly, food was measured volumetrically to the nearest 0.5 μL with a Hamilton micropipette. The fish were fed in the morning with liver paste (Gordon 1950) and in the evening with newly hatched brine shrimp nauplii. The fish were

TABLE 2. Lengths and weights after 6 and 16 wk on controlled feeding. "Age" in the first analysis refers to the 6-wk vs. 16-wk groups. ns = not significant, $p > 0.05$; *$0.01 < p < 0.05$; ***$p < 0.0001$.

A. 6 weeks

		Weight		Length	
Effect	df	SS	*F*	SS	*F*
Age[a]	1	1237.5	3.18 ns	1.4	1.2 ns
Food[b]	1	185105.6	475.8***	354.0	319.5***
Age × food	1	150.2	0.4 ns	1.4	1.3 ns
Blocks	19	14696.9	2.0 ns	27.1	1.3 ns
Age × blocks	19	5081.7	0.7 ns	14.4	0.7 ns
Food × blocks	19	6012.1	0.8 ns	10.0	0.5 ns
Residual	17	6613.8		18.8	
R^2		0.97		0.96	

Least-square means (1 SE)

Hi-6:	Hi-16:
20.3 mm (0.3), 177.5 mg (4.9)	20.3 mm (0.2), 172.1 mg (4.4)
Lo-6:	**Lo-16:**
16.2 mm (0.2), 79.5 mg (4.4)	15.6 mm (0.3), 68.3 mg (4.9)

B. 16 weeks

		Weight		Length	
Effect	df	SS	*F*	SS	*F*
Food	1	1465988.2	578.3***	760.8	1208.3***
Blocks	18	58495.3	1.3 ns	30.9	2.7*
Residual	16	40560.8		10.7	
R^2		0.98		0.99	

Least-square means (1 SE)

Hi-16:	Lo-16:
28.6 mm (0.2), 581.3 mg (12.2)	19.2 mm (0.2), 166.0 mg (12.2)

[a]Denominator for the *F*-ratio was the mean square for the time × blocks interaction.

[b]Denominator for the *F*-ratio was the mean square for the food × blocks interaction.

measured and the tanks cleaned every other week. Cleaning included a partial water change with conditioned water. Food availability was increased at these times to accommodate growth (see Table 1 for a summary of food availability). Reznick (1980, appendix 3) presented a full rationale for the choice of food rations. The tanks were inspected at least twice a day to confirm that all available food was consumed; there were only a few occasions when the entire ration was not consumed.

Fish were maintained on a 12 h light : 12 h dark photoperiod throughout their lives, with illumination provided by a Vita-Lite fluorescent bulb mounted over each row of aquaria. The temperature was maintained at 25°C and was monitored with a continuously recording thermograph with probes in the air and water. Other details of the laboratory conditions are the same as described by Reznick (1983).

Every 2 wk the fish were lightly anaesthetized in MS-222 (240 mg/L) prior to measuring them. Length was measured to the nearest 0.05 mm with vernier calipers. The fish were then blotted dry and weighed to the nearest milligram with a Mettler electrobalance.

The subjects were overdosed with anaesthetic and then preserved in 95% ethanol immediately after the last measurement.

TABLE 3. Analysis of covariance of the length of the major axis of the sagitta with standard length as the covariate. Only the Hi-6 and Lo-16 groups are compared. "LS Means" equals the mean length of the sagittus, adjusted for the effects of standard length.

A.	ANCOVA		
	df	*F*-ratio	Prob. $< p$
Covariate			
Length	1	17.9	0.0007
Main effect			
Hi-6 vs. Lo-16	1	21.7	0.0003
Blocks	19	0.44	
Residual sums of squares (df)	35.3 (15)		
R^2	0.82		

B.	LS Means (mm) (SE)
Hi-6: 0.356 (.001)	Lo-16: 0.372 (.001)

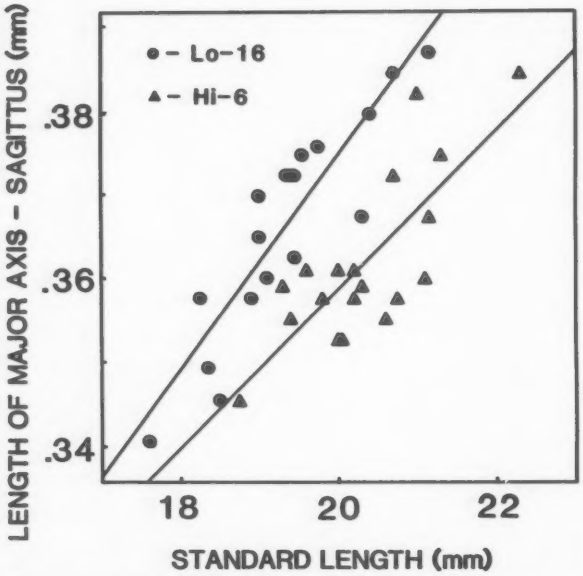

FIG. 1. Bivariate plot of the length of the major axis of the sagittus versus the standard length for the Hi-6 and Lo-16 treatments.

Otoliths were extracted and placed in a drop of immersion oil on a microscope slide. The lengths of the maximum axis of both sagittae were measured with an ocular micrometer at 200×; the two values were averaged for analysis. The individual who measured the otoliths was not familiar with the nature or expectations of the experiment.

Guppy sagittae are oval and entire and do not develop curvature as the fish ages. Some old guppies develop a more irregular outline of the otolith, including points, but this was not observed in any of the fish in this study. We previously found that the length of the major axis increases linearly with the standard length of the fish. We therefore have chosen only length of the major axis to estimate size.

All statistics were executed with the SAS General Linear Models procedure (SAS Institute 1985). There was no natural mortality during the experiment; however, one fish was discarded because it was a missexed male (Lo-16), three jumped out (2 Hi-16, 1 Lo-16), and one died during handling (Hi-6), resulting in a loss of some fish in all analyses. Type III sums of squares were used for all *F*-ratios to accommodate the missing observations. The assumptions of normality and homogeneity of variance were met for all of the reported results. In some cases, the data were log-transformed to satisfy these assumptions.

"Blocks" were included as a random effect in all analyses. This effect includes variation due to intrinsic differences among litters, plus differences resulting from variation in the age and date at initiation and in microenvironmental differences in the laboratory. Therefore, while block effects do not lend themselves to interpretation, their inclusion controls for many sources of confounding and bias and restricts comparisons to treatment differences among littermates.

Results

Effects of Food Availability on Somatic Growth

"High-food" fish grew substantially faster than "low-food" fish (Table 2B). At 6 wk, the high-food fish averaged 175 mg and 20.3 mm, as compared with 73 mg and 15.9 mm for the low-food treatment; these differences were highly significant (Table 2A). The size differences were even more dramatic when the Hi-16 and Lo-16 groups were compared at 16 wk (Table 2).

These comparisons demonstrate that the performance of the high- and low-food treatments was fairly constant throughout the 16-wk term of the experiment. This constancy reduces the chance that any differences between the Hi-6 and Lo-16 treatments are due to a factor other than age or growth rate.

Otolith Growth versus Somatic Growth Rate

The otolith dimensions in the Hi-6 and Lo-16 treatments were compared with an analysis of covariance, using the fish's standard length as the covariate. The slopes of the standard length – sagitta length regression lines did not differ between the Hi-6 and Lo-16 groups ($F_{1,33} = 1.92$, $p = 0.175$), so the analysis assumed homogenous slopes. Both "standard length" and the "treatment" effects were highly significant (Table 3A). The adjusted mean otolith lengths (LS Means in Table 3B) demonstrate that, after correcting for the effects of standard length, the Lo-16 fish had significantly larger otoliths than the Hi-6 fish by approximately 4%. The plots of individual values (Fig. 1) demonstrate that, while the magnitude of the size difference is small, there is almost no overlap between the two groups. The analysis was repeated using weight as a covariate instead of length and achieved the same results. The effect of weight was highly significant ($F_{1,19} = 13.47$, $p = 0.0023$) and the Lo-16 fish had significantly larger sagittae ($F_{1,19} = 13.96$, $p = 0.0020$) than Hi-6 fish. The magnitude of the difference was smaller, being only 3.2%.

Discussion

Implications of Otolith Scaling

We conclude that slowly growing guppies have significantly larger otoliths than equal-sized, rapidly growing guppies. These experimental results support the field observations and hypotheses proposed by Templeman and Squires (1956) and Secor and Dean (1986, 1989). The virtue of the current study is that the size differences can only be attributed to differences

in growth rate. This study does so because the genetic background of the fish, environmental conditions in the laboratory (e.g. temperature, water quality, light availability), and food quality are controlled.

Secor and Dean (1986, 1989) hypothesized that these differences in relative otolith size can be attributed to the mechanism of otolith formation (their DIP model). Briefly, they cited earlier work (reviewed by Campana and Neilson 1985) which demonstrates that daily increment formation is continuous and under endogenous control. If deposition continues, even under decreased or arrested somatic growth, the rate of increase in otolith size could exceed the rate of somatic growth, resulting in otoliths which are large relative to body size. They supported this model with their own observations on growth rate and increment formation in pond populations of striped bass (*Morone saxatilis*). A second possibility is that the sagitta maintains a constant size relative to the sacculus, but that differences in somatic growth rate are associated with subtle changes in fish shape. By this hypothesis, slowly growing fish, as compared with rapidly growing fish, would have faster growth rates in otoliths and associated structures than in length. These alternatives can be tested if the appropriate features of shape are measured in fish with different growth rates. Secor and Dean (1989) also discussed the implications of otolith scaling for the interpretation of increment width, plus the possible influence of developmental shifts (e.g. metamorphosis) on otolith scaling.

Templeman and Squires (1956) also reported that slowly growing fish have heavier and more dense otoliths than rapidly growing fish. These differences, independent of otolith length, have been attributed to (i) more slowly growing fish having a higher proportion of mineral to protein in their otoliths (Radtke et al. 1985) or (ii) an increase in otolith thickness but not length when the linear growth rate of the fish declines after maturity (reviewed by Boehlert 1985).

Differences in otolith dimensions potentially provide an index of relative growth rate which is easily obtained and could be useful in making comparisons among fish populations. More conservatively, otolith size – body size relationships could be used as an additional argument to support conclusions about growth rate obtained by other means, such as otolith ring counts. Applications could involve the use of otolith length – somatic length regression lines to make comparisons among populations, as done by Secor and Dean (1986, 1989). When applied to natural populations, the more rapid increase in otolith size should result in slowly growing populations having a steeper regression of otolith length on standard length, as opposed to the higher intercept seen in our experiment (Fig. 1), where the size range of the fish was limited. Since some fish develop curvature and marked irregularities in shape of the otolith as they age, measurements such as otolith mass may be preferred over the length measurements used in this investigation.

The differential growth rate of otoliths can also be incorporated into, and should improve, multiple regression models for age determination, such as employed by Boehlert (1985). He constructed a model for age determination based on otolith weight and linear dimensions of otoliths. A new variable to include in such models, suggested by our research, is the residual of otolith weight (or length) from the linear regression of otolith weight (or length) on fish length. If slowly growing fish have larger or heavier otoliths than rapidly growing fish, then their residuals should tend to be positive, while the residuals for rapidly growing fish should tend to be negative. These new variables have the virtue of being orthogonal to and hence independent of the size measurements.

The usefulness of relative otolith dimensions for characterizing age and growth is potentially limited by (i) the magnitude of the effect of growth rate on relative otolith size, and hence the resolution for detecting growth rate differences, (ii) the need to confirm experimentally that such growth rate – otolith size relationships are present in a given species, and (iii) the need to demonstrate that factors other than growth rate do not have a similar effect on the otolith size – body size relationship. These limitations mean that Boehlert's (1985) method and our suggested modification can only be applied with confidence in a population which has already been studied with more conventional methods. It would be most useful when applied to long-term monitoring, such as in commercial fisheries.

The difference in adjusted mean lengths for the sagittae from our two groups (4%) seems small relative to the threefold difference in growth rate (which can be inferred from the means reported in Table 2), suggesting that the resolution of any such comparisons in the field would be limited; however, the residual variances are quite small and our sample sizes are a good deal smaller than those employed in most population studies. We can demonstrate, using our data and the methods outlined by Sokal and Rohlf (1981, p. 262–264), that one can be 80% confident of detecting only a 1% difference in adjusted sagittus length at the 1% level of significance with only 70 fish in each sample. Such calculations also reveal that much smaller differences in growth rate are detectable using this methodology, providing that there is an increase in sample size. The required sample sizes fall well within those normally employed in fish population studies.

Our observed magnitude of differences in otolith size is smaller than the magnitudes observed by other authors working with different species of fish. For example, Secor and Dean (1986, 1989) found, with only a twofold difference in somatic growth rate, that individuals from a population of slowly growing fish had otoliths which were approximately 29% larger than equal-sized fish from a rapidly growing population (in the 25–30 mm size range, extrapolated from the regression equations reported with fig. 5 and 6). Where the magnitude of differences in otolith sizes is greater, the power to resolve differences in growth rate will be better.

How Prevalent is Otolith Scaling?

To evaluate the prevalence of this phenomenon, we reviewed how often the literature reports growth rate differences associated with differences in relative otolith dimensions. Other than the studies by Templeman and Squires (1956), Wilson (1984), and Secor and Dean (1986, 1989), we found equivalent information in six studies. The necessary information includes data on growth rates and otolith size – body size regressions on two or more groups of fish. The available studies are of two types: (i) observations on a natural population in two years (two studies) and ii) experiments in which treatments differed in growth rate (four studies).

Neilson et al. (1985a) and Penny and Evans (1985) both studied a natural population on two consecutive years and found that fish collected during the year with slower growth had relatively larger otoliths than fish collected during the year with more rapid growth.

Among the experimental studies, Marshall and Parker (1982, table 2 and fig. 6) reported that starved, slowly growing fish have significantly larger otoliths than equal-sized, rapidly growing fish. Temperature (less than 5°C versus 10–12°C) had no influence on this relationship. McGurk (1984, tables 2 and 4) manipulated tank volume, density, temperature, and food availability and assessed the influence of these variables on growth rate and the relationship between otolith size and body size. To evaluate our hypothesis, we correlated the slope of the regressions of body size (y-axis) on age (x-axis) with the slopes of the regressions of body size (y-axis) on otolith size (x-axis). If the predicted relationship between otolith size and body size prevails, then this correlation should be positive. The correlation is positive and marginally significant ($r = 0.697$, 6 df, $p = 0.0815$), which tends to support our prediction. The remaining two experimental studies (Geen et al. 1985; Rice et al. 1985) did not report a consistent relationship between growth rate and the relationship between otolith size and body size and hence do not support our hypothesis.

In conclusion, seven out of nine previous studies support the hypothesis that slowly growing fish have larger otoliths. All such studies carry the potential for confounding factors which could bias the results. Considering the diversity of species represented and the results of our experiment, the available data suggest that the phenomenon of slowly growing fish having relatively large otoliths is widespread. It must be emphasized that whether or not future investigators find such a relationship will depend on the size of the study and the magnitude of the differences in growth rates among the treatment groups.

Two studies reported that factors other than growth rate can affect relative otolith size. Campana (1984) found that constant light resulted in wider daily increments on the otolith; such differences should result in a larger otolith. Neilson et al. (1985b) found that incubation temperature influences the diameter of the otolith nucleus and that these size differences remained significant for 15 d after hatching. It is therefore possible for the otolith size – body size relationship to be altered by other factors. Such findings, even if they involve an unrealistic manipulation (constant photoperiod) or a temporary phenomenon (hatching diameter), suggest caution in applying this technique.

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Somatic Growth Effects on the Otolith – Fish Size Relationship in Young Pond-reared Striped Bass, *Morone saxatilis*[1]

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Somatic growth rate of pond-reared larval and juvenile striped bass, *Morone saxatilis*, influenced the relationship between otolith size and fish size. Slower growing groups of individuals had larger and heavier otoliths, relative to fish length, than did faster growing groups. Within each growth group, otolith and fish size were highly correlated. Daily increment formation was validated from 10 to 51 d after hatch. Significant interaction occurred between age and fish size effects on otolith size. We propose that otolith growth occurs by two interacting processes. Otoliths grow daily in an incremental manner which is independent of somatic growth. Growth also proceeds continuously within each daily cycle of increment deposition, probably in some proportion to daily somatic growth. Corollaries to the hypotheses are (1) somatic growth rate can influence the otolith – fish size relationship, (2) intraspecific variation in otolith scaling might be used to predict past differences in somatic growth rate, and (3) there is a biological rationale for the use of otolith size and fish size as predictors in age estimation.

Le taux de croissance somatique de larves et de juvéniles de bars rayés, *Morone saxatilis*, élevés en étangs influait sur la relation entre la taille des otolithes et celle du poisson. Les groupes de poissons à croissance plus lente présentaient des otolithes plus gros et plus lourds, par rapport à leur longueur, que les groupes de poissons à croissance plus rapide. La corrélation entre la taille des otolithes et celle des poissons était très élevée au sein des mêmes groupes de croissance. L'accroissement quotidien des otolithes a été démontré chez les individus âgés de 10 à 51 jours. Il y avait une interaction significative entre la dimension des otolithes et l'âge, d'une part, et la taille des poissons, d'autre part. Les auteurs émettent l'hypothèse que la croissance des otolithes a pour origine deux processus interactifs. Les otolithes croissent quotidiennement de par une formation de couches qui est indépendante de la croissance somatique. Il y a aussi une croissance continue, par dépôt au sein de chaque cycle quotidien, qui s'effectue probablement en proportion de la croissance somatique quotidienne. Ces hypothèses ont pour corollaires : 1) le taux de croissance somatique peut influer sur la relation entre la taille des otolithes et celle du poisson, 2) la variation intra-spécifique des anneaux des otolithes pourrait servir à déterminer l'existence d'écarts antérieurs du taux de croissance somatique et 3) l'utilisation de la taille des otolithes et des poissons en tant que paramètres d'estimation de l'âge repose sur des fondements biologiques.

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Otoliths are calcium carbonate ($CaCO_3$) concretions which occur within the labyrinth systems of fishes. A pattern of ring-like increments is commonly observed within the microstructure of otoliths (Dean et al. 1983). Over the past decade, these increments (as defined by Tanaka et al. 1981 and Wilson et al. 1983) have been verified to form with a daily periodicity in many teleosts (Campana and Neilson 1985). Recent reports of the relationship between daily somatic growth and the incremental growth of otoliths (Brothers and McFarland 1981; Lough et al. 1982; Neilson and Geen 1982; Campana 1984a; Ralston and Miyamoto 1983; Volk et al. 1984; Bradford and Geen 1987) support the use of increment widths as measures of daily somatic growth because (1) increments form daily and (2) otolith size and fish size are highly correlated. An assumption of the second criterion is that a relationship between otolith size and fish size links otolith growth to somatic growth. In this report, we model a relationship between otolith and fish size and consider whether this relationship depends upon somatic growth rate.

The term "scaling" has been applied to body size relationships (Schmidt-Nielson 1984). Here we define otolith scaling as comparisons between otolith size (e.g. otolith length, weight, area) and fish size (e.g. standard length, weight, head length). There have been several reports of intraspecific variation in otolith – fish size relationships (Table 1). Studies of the annular growth of otoliths indicate that otolith size depends upon both fish size and age (Templeman and Squires 1956; Blacker 1974; Frost and Lowry 1981; Wilson 1984; Boehlert 1985; Radtke et al. 1985). Hence, intraspecific variation in otolith scaling can be associated with varying growth rates. We hypothesize that the relationship between otolith and fish size is influenced by the daily, incremental manner in which otoliths grow.

Pond-reared larval and juvenile striped bass (life stage terms applied are those of Balon 1981) were used as a source of known-age otoliths. Pond systems were utilized to provide more natural conditions for the growth of otoliths and thus avoid the

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TABLE 1. Reports of intraspecific variation in the scaling of otolith size to fish size. Type of relationship: A = allometric, where otolith size is a nonlinear function of SL; I = isometric, where otolith size is a linear function of SL. Period of life history studied is given in parentheses: L = larval period; J = juvenile period.

Reference	Species	Type of relationship
Dean, unpubl. data[a]	*Leiostomous xanthurus*	A (L, J)
Geen et al. 1985	*Oncorhynchus tshawytscha*	A (L)
McGurk 1984	*Clupea harengus harengus*	A (L)
Marshall and Parker 1982	*Oncorhynchus nerka*	I (L)
Neilson et al. 1985a	*Oncorhynchus tshawytscha*	I (J)
Penny and Evans 1985	*Sebastes* spp.	I (L)
Rice et al. 1985	*Coregonus hoyi*	A (L), I (J)
Secor, unpubl. data[b]	*Pagrus major*	I (J)
Taubert and Coble 1977	*Lepomis macrochirus*	I (L, J)

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well-documented problems associated with otoliths from laboratory growth experiments (Campana and Neilson 1985).

Material and Methods

Experimental fish were from South Carolina's Santee–Cooper striped bass population. Striped bass eggs were stripped and hatched at the Moncks Corner Striped Bass Fish Hatchery, South Carolina, by workers of the South Carolina Wildlife and Marine Resources Department from brood stock collected in the Cooper River. Eggs of two females were fertilized with sperm of six males and mixed (genetic effects were randomized). Two such batches of fertilized eggs were prepared for two sets of ponds: one set (seven ponds) stocked on April 15, 1984, and the other set (five ponds) 11 d later on April 26, 1984. As part of the Department's fingerling production efforts, larvae were stocked without previous feeding at 250 000 per pond into twelve 1-acre (0.4-ha) earthen ponds. Ponds had been fertilized with organic and inorganic fertilizers and contained plankton blooms for the feeding larvae. No supplementary feeding occurred for any ponds.

Individuals, ages 10–55 d after hatch, were collected weekly. Larvae less than 20 mm standard length (SL) were collected at night with a strong light beam directed above a plankton net. Larger larvae and juveniles were seined. Due to low survival in certain ponds and collecting constraints, samples were unavailable or low in number for certain weeks (Table 2). Collected fish were put in ice water; they were then placed in 95% ethanol within 3 h to prevent degradation. Forty larvae and juveniles, sizes ranging from 12 to 30 mm, were measured before and after preservation to quantify shrinkage. Saccular otoliths (sagittae) were removed, cleaned, weighed, and measured along their longest axis under a dissecting microscope. As no difference could be demonstrated between the right and left otoliths, either was used (Table 3).

Preparation of otoliths for increment counts followed the procedure described by Haake et al. (1982). Otolith sections were further prepared for examination under a light microscope by gluing the polished face of the section to a thin (1 mm) plastic square (1 cm^2) with a cyano-acrylic glue and again polishing to the core. For scanning electron microscopic (SEM) examination, otolith sections were decalcified for 4–10 min in 2% EDTA (pH at 7.4) or 3–4 h in 2% glutaraldehyde (EM grade). To calibrate technique for counting increments between investigators, SEM micrographs of six sections were examined for the following microstructural features: the primordium (initial site of calcium deposition), core (area of the otolith contained within the earliest deposited increment), and discontinuous and accretion zones which together comprise one increment (terminology from Tanaka et al. 1981; Mugiya 1987). The frontal plane (i.e. section) of the otolith was chosen for increment counts, since it contained microstructural features which were most consistently identified. Ring-like features described as "subdaily increments" (Campana and Neilson 1985) sometimes occurred along major growth axes of the otolith. These features did not meet the criteria for designation as an increment. By carefully following the course of these subdaily features, it was observed that they converged with discontinuous zones of properly identified increments in lateral and medial areas of the otolith.

To validate daily increment formation, 35 otoliths which represented all sample dates from a single pond were sectioned. Increments were counted three times each by two investigators with a compound microscope at 600× magnification (resolu-

TABLE 2. Numbers of fish sampled from ponds at various days after hatch. Sample numbers collected at each age (days after hatch) are listed. Dates with 0 samples indicate sample dates when no fish could be collected. Survival was estimated at harvest (50–55 d after hatch). FG = Pond Fast Growth; SG = Pond Slow Growth.

Pond	Hatch date (April)	Survival (%)	Days after hatch																
			8	10	14	17	19	20	22	24	28	31	35	38	41	45	48	51	53	54
A	15	3	0		0			0		0		10		0		10			0	
B	15	1	0		0		0			0		0		0		10			10	
C	15	2	0		0		0			0		0		0		10			5	
FG	15	4		0		0			15			13		11		34		28		
E	15	13		4	10			0		10		7		8		10				10
F	30	48	6		0			6			10		6		9			0		
G	30	45	2			0		10			9				10		10			
H	15	33	0		0		4			10		10		10		10		10		
SG	15	46	7			3				12				38		36		36		
J	30	53	5			5		9			10			0			15			
K	30	69	4		11		0				10		0		5		10			
L	30	53	5		10			9			11			7				10		

TABLE 3. Precision estimates based on two repeated measurements of the same sample. No difference was found between repeated measurements for any of the variables ($p < 0.05$; paired t-test). N = number of paired comparisons; range = range in values of measurements.

Measurement	Mean difference	Standard deviation	Precision of instrument	N	Range
Standard length (mm)	0.10	0.013	0.05	33	12–28
Otolith length (μm)	10	2.1	20	46	517–1433
Right vs. left otolith length	11	2.2	20	31	480–1200
Increment counts					
Between readers	3.2	0.63	1	29	10–51
Within reader A	2.5	0.42	1	29	10–51
Within reader B	1.9	0.33	1	29	10–51

TABLE 4. Pond-by-pond regressions of standard length (SL, mm) on age (d after hatch) and otolith length (μm) on SL. All regressions were significant at $p < 0.005$. Estimated coefficients of variation are listed. N = sample size; Size 1 = predicted SL (mm) at 55 d; Size 2 = predicted otolith length (mm) at 25 mm SL; Size 3 = predicted otolith length at 40 mm SL (given for ponds where 40 mm was attained by time of harvest). FG = Pond Fast Growth; SG = Pond Slow Growth.

		SL vs. age					Otolith length vs. SL					
Pond	Hatch date (April)	Intercept	Slope	N	R^2	Size 1	Intercept	Slope	N	R^2	Size 2	Size 3
A	15	−27.2	1.15	19	0.90	55.9	−5.6	50.0	19	0.96	1.24	1.99
B	15	−1.93	1.00	20	0.46	53.9	134	47.2	20	0.81	1.31	2.02
C	15	−28.4	1.43	14	0.54	50.3	455	37.5	14	0.54	1.39	1.96
FG	15	−1.75	0.83	94	0.71	43.9	−320	58.3	9	0.97	1.14	2.01
E	15	−6.00	0.80	47	0.96	38.0	−176	56.9	42	0.99	1.25	2.10
F	30	5.17	0.51	36	0.79	33.2	−253	63.9	33	0.94	1.34	—
G	30	6.58	0.39	51	0.77	28.0	−401	73.0	47	0.99	1.42	—
H	15	4.04	0.41	55	0.79	26.6	−383	71.6	53	0.98	1.41	—
SG	15	3.84	0.41	139	0.73	26.4	−392	70.6	59	0.97	1.37	—
J	30	7.88	0.32	44	0.82	25.5	−355	71.6	35	0.97	1.44	—
K	30	7.17	0.33	39	0.85	25.3	−408	72.8	33	0.97	1.41	—
L	30	8.16	0.22	51	0.80	20.3	−547	88.2	51	0.95	1.66	—

TABLE 5. Between-pond comparisons (F-statistic) of regressions of standard length (SL), otolith length, and weight on age (d after hatch), of otolith length and weight on SL, and of otolith weight on length. N = sample size for Pond Fast Growth (FG) and Pond Slow Growth (SG).

Relationship	N/FG	N/SG	F	p
SL vs. age	139	94	189.4	<0.0001
Otolith length vs. age	59	39	114.1	<0.0001
Otolith weight[a] vs. age	23	25	61.8	<0.0001
Otolith length vs. SL	59	39	96.9	<0.0001
Otolith weight[a] vs. SL	23	25	84.6	<0.0001
Otolith length[b] vs. otolith weight	23	25	4.9	0.03

[a]Cubed root of otolith weight compared.
[b]Cube of otolith length compared.

tion = 1.5 μm). Otolith sections were randomized between counts. Six otoliths, for which counts varied more than 15% between readers, were omitted. Samples from the hatchery (age <6 d) were examined for first increment formation.

Precision of measured parameters was estimated as the mean of absolute differences among replicated measurements. Comparisons made between linear models used the F-test for common slope and intercept described by Ott (1977).

Results

All precision estimates were within the range of the precision of the instruments used (Table 3). SL was slightly affected by alcohol fixation (fresh SL = 0.34 + 1.037 × shrinkage SL; $n = 30$; $R^2 = 0.98$). Residuals for this relationship showed constant variance over the size range tested. Pertinent regressions were adjusted for shrinkage.

Somatic Growth Differences

Unaccounted differences occurred among the ponds with respect to their population's survival and growth rates. An event prior to stocking (e.g. handling stress, poor pond conditions) may have caused an initial mortality event in certain ponds and allowed a release from density-dependent growth for the few survivors (Secor and Dean 1986). Cannibalism might also explain some of the difference, but is not probable because all ponds were stocked at similar rates. Whatever the reason for pond-specific survival and growth rates, this fortuitous event enabled us to investigate somatic growth and age effects on otolith scaling. Pond-specific growth rates were estimated using linear regressions (Table 4). All regressions were significant ($p < 0.001$). Low coefficients of determination for ponds B and C may have been due to small sample sizes (Table 2). Predicted sizes at age 55 d (approximate harvest time) ranged from 20.3 to 55.9 mm among the ponds and were inversely related to survival estimates (Table 2). Otolith size was significantly correlated with SL ($p < 0.001$) (Table 4). To observe somatic growth effects on otolith scaling, predicted otolith sizes at 25 and 40 mm SL were calculated and arranged in descending order with respect to pond-specific growth rates (Table 4). The

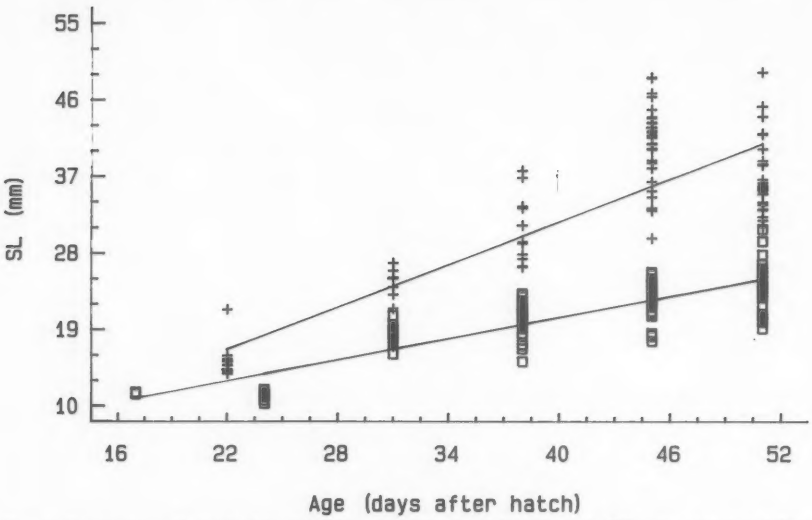

FIG. 1. Regressions of standard length (SL) on age for Pond Fast Growth (FG, plus signs) and Pond Slow Growth (SG, squares). Pond FG: SL = $-1.7 + 0.83 \times$ age; $R^2 = 0.71$; $N = 94$. Pond SG: SL = $3.8 + 0.41 \times$ age; $R^2 = 0.73$; $N = 139$.

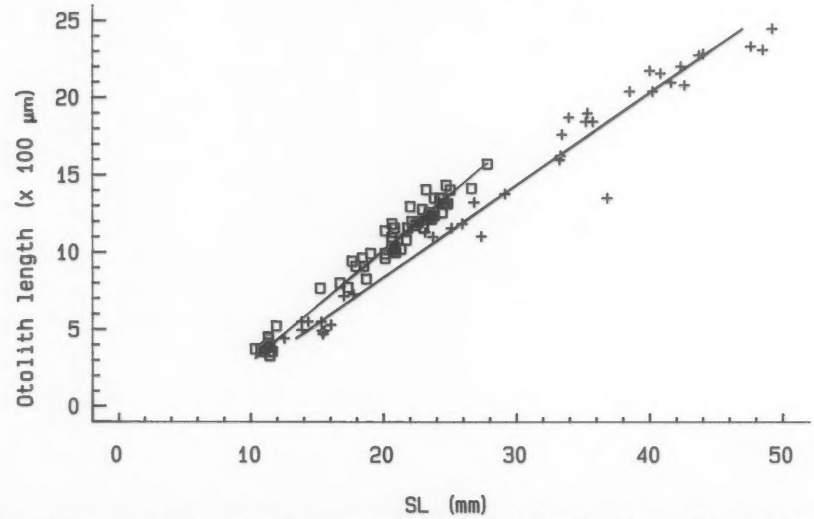

FIG. 2. Regressions of otolith length on standard length (SL) for Pond Fast Growth (FG, plus signs) and Pond Slow Growth (SG, squares). Pond FG: otolith length = $-320 + 58.3 \times$ SL; $R^2 = 0.97$; $N = 39$. Pond SG: otolith length = $-391 + 70.6 \times$ SL; $R^2 = 0.97$; $N = 59$.

trend shown is that for populations experiencing slower growth rates, otolith size was greater in proportion to fish length. The following description of how the otolith scaled to fish size and the otolith's manner of growth is presented to further elucidate somatic growth effects on otolith scaling.

Otolith Size Relationships

To simplify analysis and avoid problems in comparing ponds of different parentage and stocking dates and of low sample sizes, examination of otolith scaling was limited to two ponds, both stocked on April 15; Pond Fast Growth (FG) and Pond Slow Growth (SG) (Tables 2 and 4). Somatic and otolith growth were estimated with linear models for both ponds to permit comparisons between regressions (Ott 1977). Other models did not fit the data significantly better (von Bertalanffy: Pond 37: $R^2 = 0.72$; Pond SG: $R^2 = 0.69$; logistic: Pond FG: $R^2 = 0.79$; Pond SG: $R^2 = 0.77$; but residual variance was age dependent for both logistic regressions). Differences in somatic growth rates and otolith – fish length relationships between the two ponds were significant ($p < 0.0001$) (Table 5; Fig. 1 and 2). Otolith length inclined more steeply for Pond SG with increasing SL than for Pond FG. Hence, individuals of the same SL but from different ponds (i.e. with different growth rates) had different otolith lengths; the larger otoliths occurred for the slower growing group of fish. The change in otolith length with

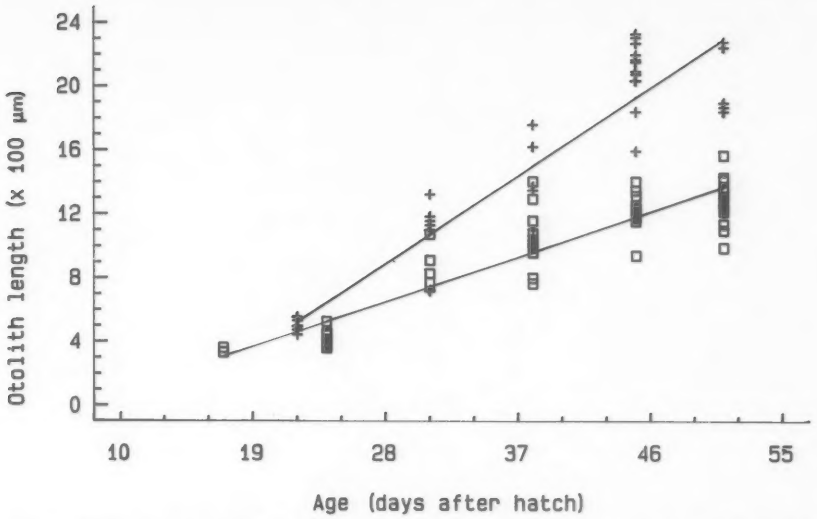

FIG. 3. Regressions of otolith length on age for Pond Fast Growth (FG, plus signs) and Pond Slow Growth (SG, squares). Pond FG: otolith length = $-830.5 + 61.3 \times$ age; $R^2 = 0.89$; $N = 39$. Pond SG: otolith length = $-184.5 + 30.3 \times$ age; $R^2 = 0.81$; $N = 59$.

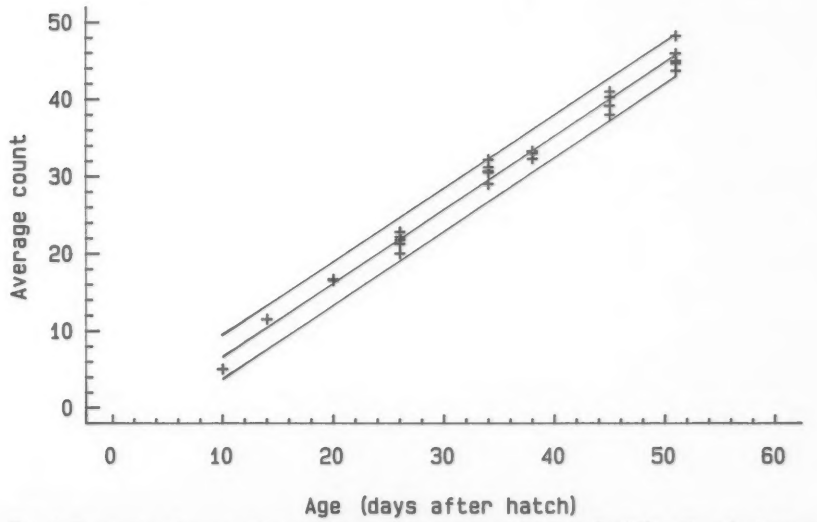

FIG. 4. Regression of average increment count on known-age of fish from Pond Slow Growth. Averages for each otolith were calculated for six repeated counts (two investigators each did three repeated counts). Prediction interval shown. Increment count = $-2.97 + 0.95 \times$ age; $R^2 = 0.99$; $N = 29$.

age complemented somatic growth differences between the ponds (Table 5; Fig. 3). Otolith weight relationships were modeled using the cube root of otolith weight, since analysis of residuals met the assumptions of constant and independent variance. Also, mass is expected to scale with the third power of length (Schmidt-Nielson 1984). The slopes of otolith weight regressed on SL and otolith length inclined more steeply for Pond SG than Pond FG (Table 5).

Assumptions for constant and independent variance of residuals were met for all regressions except for somatic and otolith growth relationships. Significant departures from constant variance occurred beyond 45 d and indicated that beyond this time, the rate of increase in SL or otolith length declined away from a linear relationship for both ponds.

Incremental Growth of Otoliths

First increment formation occurred between 4 and 5 d after hatch. Examination of the sagittae from older individuals with light microscopy and SEM showed distinct increments. Otolith material was taken from Pond SG, since some reports have indicated that nondaily increment formation occurs due to low growth rate (Geffen 1982; Lough et al. 1982; Jones 1986) although more recent reports have shown that nondaily increment formation is probably due to limited resolution (Jones and Brothers 1987; Campana et al. 1987). The number of incre-

TABLE 6. *F*-statistics and levels of significance for individual variables in otolith length and age prediction models. All regressions were significant at $p = 0.0001$ ($N = 455$). S = significant at $p<0.001$; MS = marginally significant at $0.1 > p > 0.01$; NS = nonsignificant at $p > 0.1$; P = pond; H = hatch date; Otol = otolith length.

Otolith length =							
SL	Age	SL × age	Pond	Hatch date	SL × age × P	SL × age × H	R^2
584 S	275 S	23.0 S	—	—	—	—	0.97
800 S	212 S	5.6 MS	39.1 S	—	0.70 NS	—	0.98
713 S	226 S	22.3 S	—	28.7 S	—	0.39 NS	0.98

Age =							
SL	Otol	SL × Otol	Pond	Hatch date	SL × Otol × P	SL × Otol × H	R^2
0.27 NS	381 S	99.7 S	—	—	—	—	0.81
4.29 MS	301 S	63.7 S	29.1 S	—	24.9 S	—	0.82
10.9 S	346 S	102.4 S	—	71.9 S	—	16.0 S	0.84

ments counted for each otolith was not influenced by the order in which the otolith was read ($p = 0.61$), nor were they significantly different between readers ($p = 0.50$). Increment counts for each otolith were averaged and regressed against age (Fig. 4). The slope was not different from the expected rate of one increment per day, nor was the expected intercept significantly different than the modeled intercept based on observed first increment formation at 4–5 d after hatch (average number of increments $= -2.96 + 0.95 \times$ d after hatch; *F*-test for slope of 1 and intercept of -4, $p = 0.08$).

Otolith Scaling Model

To assess the generality of somatic growth effects on otolith scaling, otolith length was modeled with data pooled from all 12 ponds (Table 6). A model which used age and SL alone showed significant main effects and interaction for these variables (Table 6). Other models which included pond and hatch date effects showed significant main effects due to the additional variables but improved prediction of otolith length negligibly. Also, no significant interaction occurred between the variables SL, age, and pond or hatch date.

Age prediction models were fit using otolith length, SL, pond, and hatch date effects (Table 6). Main effects for SL were significant only when the model included hatch date or pond effects. Pond and hatch date effects, albeit significant, did little to improve the age prediction regression. Age prediction models using SL and the cube root of otolith weight were tested for combined data from Pond FG and Pond SG ($n = 48$). Main effects (cube root of otolith weight and SL) and interaction terms (cube root of otolith weight × SL and cube root of otolith weight × SL × pond) were significant at $p = 0.0001$ with a high determination coefficient ($R^2 = 0.91$).

Discussion

DIP Model

We show that differences in otolith scaling patterns were attributable to somatic growth differences. Since scaling differences occurred between ponds (Pond FG versus Pond SG) which were stocked with larvae from the same batch of fertilized eggs, temporal and genetic differences can be discounted. For pooled data, nonsignificant effects of pond and hatch date on the interaction term SL × age also suggest that location, genetic, and temporal influences can be ruled out. Based on the results, we propose a model for otolith size which considers both fish size and age effects. We believe that this model is appropriate, since daily increment formation (i.e. age effects) can occur independent of somatic growth. Because increment formation will always contribute to otolith growth in the model, we refer to it as the daily increment packing (DIP) model for otolith scaling.

Uncoupling Hypothesis

Evidence suggests that daily accretion of $CaCO_3$ and organic matrix onto the otolith is an endogenously defined rhythm (Mugiya et al. 1981; Radtke and Dean 1982; and many others, see Campana and Neilson 1985). Therefore it is possible for changes in otolith size to occur that are not synchronous with changes in fish size. In an extreme case, cessation of fish growth could occur and the increment growth of the otolith continue, resulting in dissociation or uncoupling of otolith and somatic growth. Uncoupling has been used to explain intraspecific variation in otolith scaling. Uncoupling may be due to differences in core size (Neilson et al. 1985b), experimental manipulation of photoperiod (Campana 1984b), metamorphic transition (Brothers and McFarland 1981; Nishimura and Yamada 1984), or cessation of growth (Brothers 1981; Marshall and Parker 1982; Campana 1984a). For this study, analysis of residuals for somatic and otolith growth regressions showed a declination from linearity after 45 d. But previous to the last week of the sampling period, somatic and otolith growth was continuous. A comparison between Pond FG and Pond SG with all samples older than 45 d omitted showed signficant differences in the otolith length – SL relationship ($n = 79$; $F = 69.2$; $p = 0.0001$). Also, no inflection point in the otolith length – SL relationshp was observed which corresponded to the shift in growth rate (Fig. 2). Our evidence suggests that fish growth need not be interrupted for differences in otolith scaling to occur. Uncoupling probably represents one extreme in a continuum of scaling relationships influenced by somatic growth rate.

Rationale for the DIP Model

A range of somatic growth effects on otolith scaling can be explained by two interacting components of increment deposition, a temporal component and an amplitude component.

Mugiya (1987) has shown through in vitro studies that otolith growth depends upon diurnal cycles of $CaCO_3$ and organic matrix deposition, each cycle occurring in opposite phase with the other (e.g. the peak of $CaCO_3$ deposition is simultaneous to the nadir of organic matrix deposition and vice versa). Since otoliths are primarily mineral (Degens et al. 1969), we will apply further arguments to $CaCO_3$ deposition only. Diurnal variation of $CaCO_3$ deposition onto the otolith and serum calcium were shown to be simultaneous (Mugiya et al. 1981; Mugiya 1987). Wagner et al. (1985) recorded variation in daily calcium uptake rates for rainbow trout (*Salmo gairdneri*) fry which cycled every 7–17 d. They indirectly related this variation to cycles in weight gain. Assuming that calcium uptake and serum calcium level are related (Taylor 1985; Wagner et al. 1985), there is biological rationale for relating changes in fish length (or weight) to otolith growth. Conceptually, daily changes in fish size are associated with the amplitude of a deposition period of $CaCO_3$ (i.e. the area under a peak). Otolith growth as defined by deposition period, and each period's amplitude can thus be related to age and fish size.

The DIP model assumes that increment formation is only related to age. A few studies have concluded that somatic growth rate influences increment formation (Geffen 1982, 1983; McGurk 1984). In a recent study using SEM (Jones and Brothers 1987), daily increment formation was confirmed for larval striped bass reared in aquaria and starved intermittently. It has been suggested that resolution and otolith preparation may limit the ability to observe increments in instances of reduced fish growth (Campana and Neilson 1985; Jones and Brothers 1987; Campana et al. 1987).

Several reports of intraspecific variation in otolith scaling have suggested growth- and age-related causes. Based on a recent a priori test on age-related otolith – fish length differences, Reznick et al. (1989) concluded that although age-related differences in otolith size were small in magnitude, they were still statistically significant. In a similar experiment, Marshall and Parker (1982) found that fish on a lower ration level grew less and yielded larger otoliths at a given fish length. However, Rice et al. (1985) showed that variation in scaling was not attributed to somatic growth rate (ration). More descriptive field studies relate intraspecific variation in otolith scaling to year-class (Templeman and Squire 1956; Neilson et al. 1985a; Penny and Evans 1985), cohorts (Messieh 1972), sampling locations (Taubert and Coble 1977; Frost and Lowry 1981), and sex (Templeman and Squires 1956; Wilson 1984). It is feasible that these examples of intraspecific variation may have occurred due to somatic growth effects. For instance, Wilson (1984) provided strong evidence for a scaling – somatic growth relationship in adult billfishes. Sexual dimorphism in fish size resulted from discrete sex-related growth rates. Otoliths were significantly heavier for older males than for younger females at the same body weight. Templeman and Squires (1956) described similar results for adult haddock (*Melanogrammus aeglefinus*). Because the daily deposition of $CaCO_3$ and organic matrix determines otolith growth in adult fishes (Taubert and Coble 1977; Mugiya et al. 1981; Wilson 1984; Mugiya 1987), these cases of growth-related variation in otolith scaling for adults are relevant to the DIP model.

Age Prediction based on the DIP Model

Since otolith growth depends upon the period of $CaCO_3$ deposition as well as the amplitude of each deposition period, age and SL effects on otolith size were expected. By rearranging these variables, we were able to predict age based on otolith size and fish size. Conceptually, were growth rates not variable within a population, then otolith size would only depend upon age (period of deposition). But variable growth rates, manifested in SL, will affect daily deposition rates (period amplitude). Therefore, SL will influence the relationship between age and otolith length. Hence, the regression model for age had significant interaction between SL and otolith length. But SL main effects were not always significant. This may be due to colinearity between otolith length and SL.

Reznick et al. (1989) suggested that the residuals from the regression otolith length = SL may be used in age prediction models. We tested several combinations of this term and found that the best prediction equation was age = otolith length + (otolith length − residual). With $R^2 = 0.76$, it explained less variation than a model based on interaction between otolith and fish length.

Implications for Interpretation of Increment Widths

Several workers have used increment widths as estimators of age-specific growth rates (see the introduction). These studies assume a simple relationship between otolith and fish size which is species specific. Our results suggest a more complex relationship. (1) Within a species, there are population-specific otolith scaling patterns which are related to growth rates. Therefore for each population studied, an otolith – fish size relationship must be verified. (2) Among individual fish, increment widths will be a function of the period of deposition and that period's amplitude. For instance on a day when somatic growth is negative, zero, or very slow, some minimum amount of $CaCO_3$ and organic matrix is deposited on the otolith (Marshall and Parker 1982; Jones and Brothers 1987). On a day when growth is fair to good, material additional to this minimum amount is deposited on the otolith. The actual relationship between increment width and somatic growth is much more complicated than this; beyond the minimum limit, increment width is probably a declining function of somatic growth rate (Baldeverona 1987). Therefore, one cannot assume that increment width and somatic growth are linearly related; minimum and maximum limits on increment widths will have to be integrated into any age-specific growth rate model. A further complication is that otoliths change shape throughout ontogenesis (Brothers and McFarland 1981; Campana 1984a; Nishimura and Yamada 1984). As otolith growth axes shift, so will the minimum and maximum limits.

Limitations of the DIP Model

Factors other than somatic growth rate can be associated with differences in scaling and may curtail prediction of somatic growth rate and age. These include factors which effect uncoupling (see previous discussion), the $CaCO_3$ to organic matrix ratio (Radtke et al. 1985), life history events (Brothers and McFarland 1981; Campana 1984a; Nishimura and Yamada 1984), temperature, genotypic variation, and variable growth rates. Radtke et al. (1985) suggested that the protein to $CaCO_3$ ratio may vary according to growth rate. Fast growing fish would deposit more protein relative to $CaCO_3$, resulting in less dense, lighter otoliths. This hypothesis is speculative, since protein content in otoliths has been rarely quantified. Degens et al. (1969) measured organic content for otoliths of 25 species which ranged from 0.25 to 10.14% and averaged 1.5%. Tem-

perature will affect the amplitude of the deposition period (Y. Mugiya, Faculty of Fisheries, Hokkaido University, Minato-3, Hakodate, 041 Japan, pers. comm.). Should populations be exposed to variable temperatures, this factor may complicate predictions based on scaling. In this study, genotypic variation was randomized by mixing batches of eggs. Until genotypic variation is quantified, the use of otolith scaling should be confined to population studies.

Age and growth rate prediction on the basis of otolith scaling assumes a constant growth rate for both the fish and the otolith. Both of these assumptions limit application, since somatic growth rate is known to change throughout a fish's life history due to such events as starvation or compensatory growth. Also, otoliths will undergo metamorphic shifts as they grow, resulting in shifts in growth axes. Therefore we do not propose otolith scaling as a precise measure, but rather as an index or an average of past growth rates. The index will be limited to population studies which consider discrete life stages and assume constant growth rates (D. Reznick et al. 1989).

Future Studies

In the future, it may be possible to predict relative differences in somatic growth between groups of young-of-the-year fishes with otolith size relationships (Secor and Dean 1986). Prediction intervals for the otolith length – SL relationship for Pond FG and Pond SG (not shown) overlapped due to the range in data for each growth group (Fig. 2). However, with further testing and modification, the DIP model may allow fishery workers to deduce that a larger otolith, from a comparison of fish of the same length, is indicative of slower growth. As we have demonstrated through a statistical and conceptual model, age prediction on the basis of otolith scaling is also feasible. Otolith weight may be a better dimension of otolith growth and hence allow better age prediction. Otolith – fish size relationships are easily measured, especially compared with the arduous tasks of increment counts and increment width measures. Comparisons could be made between hatchery and naturally produced fish, spawning sites, and year-classes.

Future studies need to establish a physiological link between fish anabolism and otolith growth. Mugiya (1986) has shown that the regulatory protein calmodulin (which is regulated by serum calcium) is involved in otolithic calcification; but a regulatory sequence from general anabolic activity to $CaCO_3$ deposition remains to be elucidated. Integral problems to investigate include the role of organic matrix in $CaCO_3$ deposition and the function of the pineal and pituitary glands and their hormones on the deposition of organic matrix and $CaCO_3$.

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Cardiac Responses to a Natural Odorant as Evidence of a Sensitive Period for Olfactory Imprinting in Young Atlantic Salmon, *Salmo salar*[1]

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Olfactory imprinting was assessed in young Atlantic salmon, *Salmo salar*, undergoing smoltification (parr–smolt transition) by measuring their cardiac responses to a natural odorant, L-cysteine. Condition factor and body coloration were used for characterizing the degree of smoltification. In Experiment 1, heart rate conditioning to L-cysteine was used to compare olfactory learning between fish from different age groups of smoltification. In Experiments 2 and 3, other fish from the same age groups of smoltification were exposed to L-cysteine and their long-term olfactory memory was assessed by measuring their unconditioned cardiac responses to L-cysteine after smoltification. In Experiment 2, the time from the end of odor exposure to testing for olfactory recognition was kept constant for all age groups of smoltification whereas in Experiment 3, the age of fish tested for olfactory recognition was kept constant. Greater conditioning (heart rate reduction) to L-cysteine occurred in age-groups 3 (612–619 d since birth) and 6 (642–649 d) as compared with any other age group of smoltification. Fish tested for odor recognition exhibited a greater unconditioned response (cardiac deceleration) to L-cysteine if they belonged to age-group 3 than to any other age group of smoltification. Our results demonstrated the existence of a sensitive period for olfactory imprinting in Atlantic salmon that occurred between 21 and 28 d after the onset of smoltification induced in the laboratory.

Nous avons évalué l'empreinte olfactive chez des jeunes saumons atlantiques, *Salmo salar*, en smoltification (transition tacon-saumoneau) en mesurant leur réponse cardiaque à une substance odorante naturelle, la L-cystéine. Le facteur de condition et la coloration corporelle sont utilisés pour caractériser le degré de smoltification. Dans l'Expérience 1, le conditionnement de la fréquence cardiaque à la L-cystéine est utilisé pour comparer l'apprentissage olfactif entre des poissons de différents groupes d'âge en smoltification. Dans les Expériences 2 et 3, d'autres poissons des mêmes groupes d'âge en smoltification sont exposés à la L-cystéine et leur mémoire olfactive à long terme est évaluée en mesurant leur réponse cardiaque inconditionnelle à la L-cystéine après la smoltification. Dans l'Expérience 2, l'intervalle de temps entre la fin de l'exposition à l'odeur et le test de la reconnaissance olfactive était maintenu constant pour tous les groupes d'âge à la smoltification alors que dans l'Expérience 3, l'âge des poissons testés pour la reconnaissance olfactive était maintenu constant. Un conditionnement (réduction de la fréquence cardiaque) plus marqué est apparu chez les groupes d'âge 3 (612–619 d depuis la naissance) et 6 (642–649 d) par rapport à n'importe quel autre groupe d'âge de la smoltification. Les poissons testés pour la reconnaissance olfactive et appartenant au groupe d'âge 3 ont manifesté une plus forte résponse inconditionnelle (décélération cardiaque) à la L-cystéine que n'importe quel autre groupe d'âge de la smoltification. Nos résultats démontrent l'existence d'une période sensible d'empreinte olfactive chez le saumon atlantique qui s'est produite entre 21 et 28 d après le début de la smoltification induite en laboratoire.

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Imprinting is a well-known phenomenon in higher vertebrates, especially in precocial birds (Hess 1973). In the few hours after hatching or birth, offspring acquire a following response to their parents or to artificial stimuli resembling them. This response, which is the basis of a strong attachment bond with the imprinting stimulus, results from a learning process that takes place during a restricted period in an individual's life known as the sensitive period. Imprinting results in relatively long-lasting memory and may be completed at a time when the appropriate reaction is itself not yet performed (Immelmann and Suomi 1981). Although the concept of imprinting and the validity of some of the aforementioned criteria are controversial

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issues, in all phenomena that have been characterized as imprinting, some learning processes are favored during specific, usually early, periods of the individual's life time (Immelmann and Suomi 1981).

Attempts have been made to identify various kinds of imprinting in lower vertebrates such as turtles (e.g. Grassman et al. 1984) and fish (e.g. Russock 1986) but the sensitive period has generally proved difficult to define. In anadromous salmonids, homing behavior has been attributed to an olfactory imprinting mechanism. Adult salmon would be able to return to their spawning grounds by detecting and orienting toward the natal stream odor that they imprinted to as young fish.

Supporting evidence for a long-term olfactory memory in salmon comes from artificial imprinting studies. Prior to seaward migration, young coho salmon, *Oncorhynchus kisutch*, exposed to the synthetic chemical morpholine were subsequently decoyed as adults into streams scented with this same artificial chemical (Hasler and Scholz 1983). Although these studies have established the olfactory hypothesis as the leading explanation for homestream recognition by homing salmon, an important feature of true imprinting has still to be demonstrated: the existence, onset, and duration of a sensitive period for olfactory imprinting (SPOI) (Hara 1970; Hess 1973; McCleave et al. 1984; Stabell 1984). At this point, it is worth noting that imprinting necessarily implies a long-term retention of what is learned during the sensitive period but any long-term memory phenomenon is not always the result of an imprinting process.

Classical experiments on imprinting in birds have determined the temporal limits of the sensitive period by exposing groups of young birds to natural or to artificial stimuli during identical short time periods during different developmental phases (Hess 1973). This experimental approach has not been used in any study of salmon imprinting. However, attempts have been made to define the SPOI by exposing groups of young salmon to artificial odorous chemicals during smoltification. The parr–smolt transition is generally believed to be the most probable developmental stage for the SPOI even though a sensitive period occurring during earlier life history stages (including the egg stage) is not excluded (Horrall 1981; Quinn 1985).

A period ranging from 4 to 9 wk, coincident with smoltification, has been used for imprinting salmonids to an artificial chemical (Dizon et al. 1973; Madison et al. 1973; Scholz et al. 1973; Cooper and Hasler 1973, 1974, 1976; Cooper and Scholz 1976; Cooper et al. 1976; Scholz et al. 1976; Scholz et al. 1978a, 1978b; Johnsen and Hasler 1980). Time periods of less than 4 d have occasionally been used for imprinting (Cooper et al. 1976; Sutterlin et al. 1982). In one study (Cooper et al. 1976), a group of coho salmon undergoing smoltification was exposed to morpholine during 44 d while another group was exposed to the same chemical for 2 d. They were released in Lake Michigan and later caught during two consecutive years in a stream scented with morpholine. Results revealed, quite surprisingly, that more fish from the 2-d exposure group (n = 78) were captured in the morpholine-scented stream than fish from the 44-d exposure group (n = 46). Similar inconsistencies between morpholine exposure time and frequency of migrant returns have been found in other imprinting studies (e.g. Madison et al. 1973; Sutterlin et al. 1982).

Although they suggested that olfactory learning during smoltification is a relatively rapid process, previous investigations have failed to accurately define the SPOI. It has only been roughly estimated from a small number of adults returning to an artificially scented stream. Therefore, any link between the classical phenomenon of filial imprinting in precocial birds and long-term olfactory memory in salmonids is still speculative and must be substantiated.

Fish are known to decelerate heart rate readily to various novel environmental stimuli (Laming and Savage 1980) including sound (Hawkins and Johnstone 1978), light (Kawamura et al. 1981), temperature (Nagai and Iriki 1977), and chemical substances (Malyukina and Martem'yanov 1981). In young salmonids, cardiac deceleration has been used as an unconditioned response to assess olfactory memory (Muzi, cited in Hasler and Scholz 1983) and also as a conditioned response (associative learning) to assess their ability for detecting odors (Hirsch 1977; Sandoval 1980; Morin et al. 1987a, 1987b).

The purpose of this study is to verify, in young Atlantic salmon, *Salmo salar*, undergoing smoltification, the validity of the imprinting theory as an explanation of long-term olfactory memory in homing adult salmon. Three experiments were conducted to define when the sensitive period occurs during smoltification and to reveal that a sensitive period had occurred by its effects on olfactory recognition following smoltification. In the first experiment, the magnitude of a conditioned cardiac deceleration to a natural odorant, L-cysteine, was measured in fish from different age groups of smoltification. The sensitive period for imprinting begins when a stimulus becomes conspicuous to an animal (Bateson 1964). This should facilitate the acquisition of a conditioned response. We therefore postulated that the ability to learn an odor should be optimal during a sensitive period and that the magnitude of the conditioned cardiac deceleration would significantly increase at such times.

In the second and third experiments, retention of an olfactory memory was evaluated by simply exposing fish to L-cysteine at different time intervals during smoltification and measuring their unconditioned cardiac deceleration to the odor at different intervals after smoltification. Fish decelerate heart rate in response to a recognized odor. Young coho salmon decelerated heart rate in the presence of an imprinted odor (morpholine or phenethyl alcohol) 85–100% of the time as compared with 0–10% in the presence of a novel odor (Hasler and Scholz 1983). We therefore postulated that the magnitude of unconditioned cardiac deceleration would be significantly greater among fish exposed to an odor during a sensitive period. In Experiment 2, the time from the end of odor exposure to testing for olfactory recognition was kept constant for all age groups of smoltification whereas in Experiment 3, the age of fish tested for olfactory recognition was kept constant. This was done to avoid confounding the influence of the age of testing and time between exposure and testing (Bateson and Hinde 1987).

Materials and Methods

Fish and Maintenance

Two hundred and forty 2-yr-old Atlantic salmon (mean fork length = 15.7 cm ± 0.8 SD) were used in the experiments. They were hatchery-raised offspring of a wild stock obtained from the Ste-Anne River, located on the Quebec north shore 370 km to the northeast of Quebec city. Fish were transported to the Aquarium du Québec and housed in a 3000-L (226.1 × 112.4 × 101.6 cm) concrete holding tank supplied with dechlorinated water (4 ± 0.5°C) under a constant 16 h dark : 8 h light photoperiod regime. The tank was completely covered and the only light sources were four incandescent 7-W bulbs situated 35 cm above the water's surface. A 300-L tank (157.5

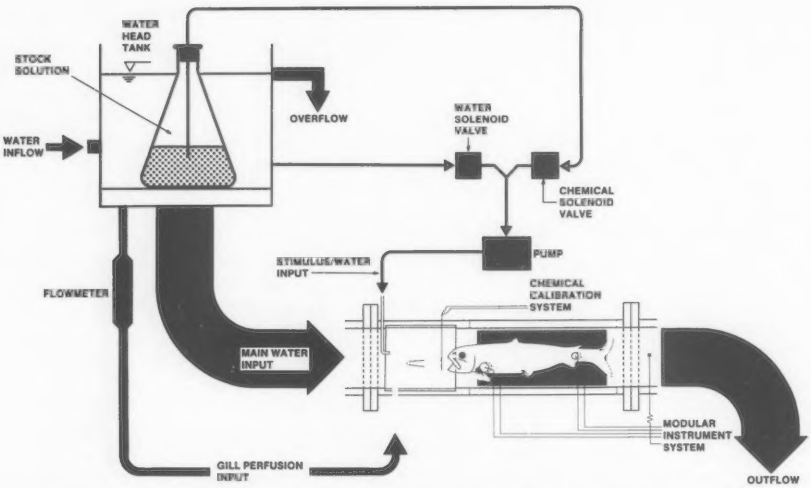

FIG. 1. Experimental layout with view of test chamber. This diagram also illustrates stimulating and cardiac monitoring electrodes attached to the fish and communicating to the modular instrument system.

× 50.8 × 35.6 cm) was used for exposing small groups of fish to an odorous substance, L-cysteine. Anosmic fish show no conditioned cardiac response to L-cysteine (unpubl. data). Photoperiod and water conditions in the 300-L tank were similar to those maintained in the 3000-L tank. All fish were fed daily with a mixture of shrimps, crab meat, and scallops. After 20 d of acclimation, 120 fish were randomly selected for odor-recognition tests. They were divided equally into six floating cages (68.6 × 34.3 × 30.5 cm) located in the holding tank. Cages were made of polyester material with "Beehive" 0.75 × 0.25 cm mesh (Gerrard Mills Inc.) and small PVC piping. The remaining fish were left to swim freely in the holding tank.

Smoltification was induced in fish by acclimating them to 11 ± 0.5°C and a long and constant 8 h dark : 16 h light photoperiod by 1° and 1-h daily increments between 23 and 29 December 1985. Natural smoltification occurs in about 30% of 2-yr-old salmon native to the Ste-Anne River (Anonymous 1987). In the laboratory, parr–smolt transition began on 30 December and was completed on 25 February for a total smoltification period of 58 d.

Apparatus

Heart rate responses of fish to an odorous chemical were assessed in a test chamber connected to different water lines and computer-assisted electronic systems (Fig. 1). This procedure is fully described in Morin et al. (1987b). The main water line contained dechlorinated water flowing at a constant velocity (20.5 mm/s) from the head tank to the test chamber before its evacuation through an outlet pipe. The stimulus-input line to the test chamber delivered either water from the head tank or a chemical stimulus from an erlenmeyer flask containing the stimulus solution placed in the head tank. Water temperature in the head tank as well as in the test chamber remained constant (10.5 ± 0.5°C) for the entire study. A peristaltic pump was used for stimulus injection which was controlled by a pair of solenoid valves. The gill-perfusion line brought water from the head tank directly to the gills of fish (300 mL/min).

The head section of the test chamber housed stainless steel and glass parts to allow free flowing of odorous molecules which could otherwise stick to walls. The test chamber was also equipped with immobilizing devices to hold the fish in position for adequate monitoring and stimulation. Electrodes used for delivering the conditioning shock to the fish or for monitoring cardiac rhythm were connected to a modular instrument system, while those used for chemodetection were connected to a chemical calibration system. Both electronic systems interacted with a microcomputer which controlled all events of an experimental session. The test chamber was located in a black box for eliminating any external visual disturbance.

Experiment 1

The magnitude of conditioned cardiac deceleration of L-cysteine was measured in fish during six successive 8-d intervals of smoltification. The age since birth of each age group was as follows: age-group 1: 592–599 d, age-group 2: 602–609 d, age-group 3: 612–619 d, age-group 4: 622–629 d, age-group 5: 632–639 d, age-group 6: 642–649 d.

Twenty fish of each age group were conditioned. As it took about 3 h to test each fish over a period of 2 d, an 8-d interval was the minimum time required to test 20 fish. A 2-d off interval separated each age group.

Parr coloration and condition factor (weight·fork length^{-3}) were used as indexes of smoltification. Parr coloration was assessed by attributing a score to fish on the presence of body marks characteristic of parr and the absence of marks characteristic of smolts. The marks and their respective scores were as follows: presence of vertical parr marks (2 points), presence of contrasting dark back and yellowish belly (1 point), presence of red spots near the lateral line (1 point), absence of blackened pectoral fins (1 point) and caudal fin (1 point), and absence of a pectoral black spot on either side of the fish (1 point). Individual fish were scored prior to conditioning and scores were averaged to generate a mean parr coloration index for each age group of smoltification.

Each fish was anesthetized (1% methyl pentynol), injected in the dorsal musculature with *d*-tubocurarine chloride (0.002 mg/g body weight), and placed in the test chamber. Injection of small quantities of tubocurarine does not interfere with learn-

ing or with odor sensitivity (Morin et al. 1987a). An experimental session commenced with a 35-min acclimatization period during which we measured resting heart rate. This was followed by a trial during which 7.3 mL of L-cysteine (5.7×10^{-3} M) was injected into the test chamber. When the stimulus reached the fish, it was diluted to a concentration of 3.8×10^{-4} M (Morin et al. 1987b). Eight seconds later, a 2-s continuous shock stimulus was delivered to fish. Intensity of stimulation (30–46 mA) was adjusted at the beginning of the session to produce strong bradycardia averaging 10 beats/min. Fish were trained over two sessions (intersession interval = 24 h) of 20 trials each, separated by short intertrial intervals of random duration (45–135 s).

Each age group of 20 fish was divided equally in two groups, each of which was treated differently on the basis of stimulus presentation. Fish from the conditioning groups were presented repeatedly with the systematic pairing of an odorous stimulus shortly followed by an electric shock. Since the odor signals the presence of the shock, this perfect correlation between the stimuli typically induces an increase in the magnitude of the conditioned response (Weisman and Litner 1969). Fish from the random pairing group were trained with a random presentation of the odor–shock sequence and were used as a control for pseudoconditioning or sensitization (Rescorla 1967). In this group, the conditioned response does not occur, since the presence of the odor is uncorrelated with that of the shock. This treatment therefore represents a good control procedure with which to assess excitatory conditioning (Mackintosh 1983).

Heart rate reduction to an odor cue typically occurs in the context of a single interbeat interval (IBI) and the phenomenon is masked when IBIs measured during the entire odor–stimulation interval are averaged. For this reason, the largest IBI recorded 8 s before odor presentation was compared with the largest IBI recorded 8 s during odor stimulation, and the percentage of heart rate reduction was calculated according to the following formula:

$$100 - \frac{\text{largest IBI before} \times 100}{\text{largest IBI during}}.$$

Learning occurred if the percentage of heart rate reduction of conditioned fish increased significantly between blocks of trials and if cardiac deceleration of conditioned fish was significantly greater than that of fish tested under the random pairing procedure (Morin et al. 1987a, 1988).

Experiments 2 and 3

In these experiments, long-term olfactory memory was assessed by measuring unconditioned cardiac responses to L-cysteine after fish from six different age groups had been exposed to an odorous chemical during smoltification. These age groups were identical to those of Experiment 1. They were exposed to L-cysteine at the same time as the subjects of Experiment 1 were conditioned to L-cysteine. Smoltification was induced in all fish as previously described.

On the first day of odor exposure, fish were transferred to a tank in which L-cysteine was diluted to obtain a concentration of 10^{-4} M. Water was renewed completely the following day (day 2). L-cysteine was added to water on days 3, 5, and 7 and water renewal occurred on days 4, 6, and 8. Fish were transferred back to their holding tank on day 8. Before exposing fish from another age group of smoltification, water in the odor–exposure tank was renewed on days 9 and 10.

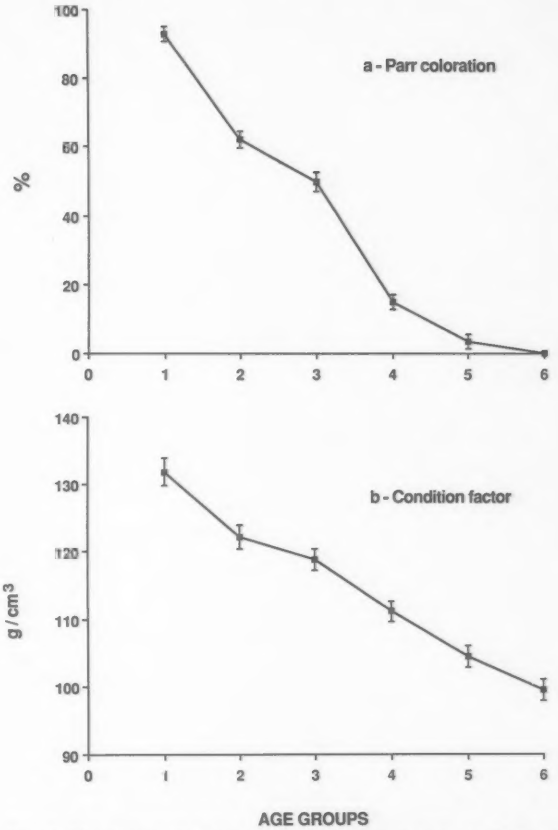

FIG. 2. Morphological changes during smoltification in six different age groups of 20 fish. (a) Parr coloration: mean percentages of body marks characterizing a parr; (b) condition factor: weight·fork length^{-3} ($\times 10^{-4}$). The vertical bars represent the mean ± SE.

Olfactory recognition was assessed in fish that had been exposed to L-cysteine during smoltification by measuring their unconditioned cardiac responses to L-cysteine at different times during the postsmoltification period. Fish from each age group of smoltification were divided equally in two groups ($n = 10$) to be tested either under a fixed- or a variable-interval treatment. Under the fixed-interval treatment (Experiment 2), fish from each age group were tested 55 d after the end of their initial exposure to L-cysteine. In these fish, the time from the end of odor exposure to testing for olfactory recognition was kept constant. Under the variable-interval treatment (Experiment 3), age-groups 1–6 were tested 85, 75, 65, 55, 45, and 35 d, respectively, after the end of their initial odor exposure. In this experiment, the age of fish tested for olfactory recognition was kept constant.

Fish were anesthetized (1% methyl pentynol), injected in the dorsal musculature with *d*-tubocurarine chloride (0.002 mg/g body weight) and placed in the test chamber for a period of 35 min before exposure trials commenced. A trial consisted of injecting 7.3 mL of L-cysteine (5.7×10^{-3}M) into the test chamber and measuring heart rate 8 s before and 8 s during odor stimulation. Applying a dilution factor calculated during a chemical calibration test (Morin et al. 1987b), we estimated that fish were exposed to a concentration of 3.8×10^{-4}M. Fish

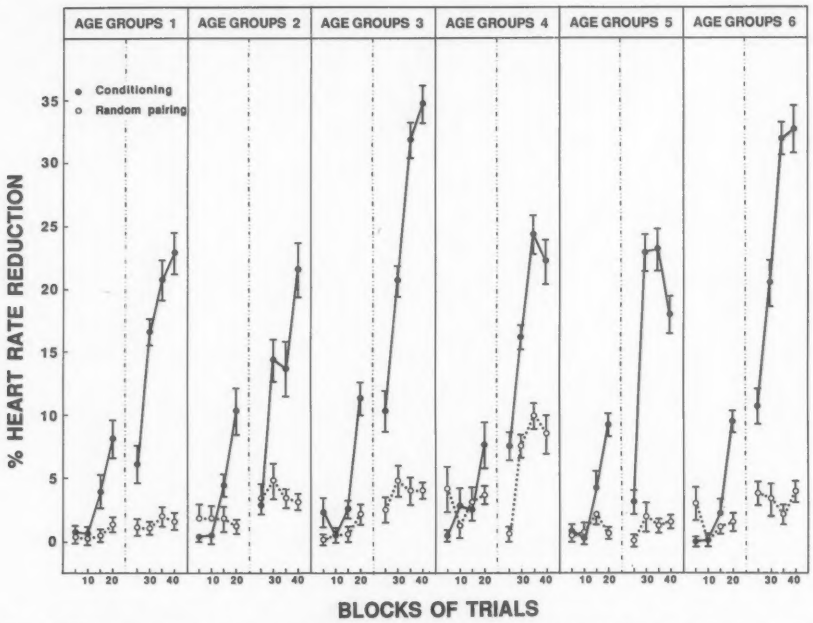

FIG. 3. Mean percentage of heart rate reduction, per block of five trials, in 2-yr-old Atlantic salmon tested in the presence of L-cysteine (3.8×10^{-4} M). During smoltification, six different age groups of 10 fish were conditioned to L-cysteine followed by a shock stimulus and six age groups of 10 fish were exposed to a random odor–shock presentation. The vertical bars represent the mean ± SE.

were tested over a single experimental session of 10 trials separated by short intertrial intervals of random duration (45–135 s). Initial cardiac rhythm and heart rate reductions to L-cysteine were assessed in the same manner as in Experiment 1.

Results

Morphological Changes during Smoltification

Changes in parr coloration during smoltification (Fig. 2) were highly correlated with changes in condition factor ($r = 0.82$). Overall differences in parr coloration ($F = 313.05$; df = 5,114; $P < 0.01$) and in condition factor ($F = 74.97$; df = 5,114; $P < 0.01$) were significant between the age groups of smoltification as both indexes declined from age-groups 1–6. The importance of these changes was also examined between successive age groups. All fish from age-group 1 could be distinctively identified as parr. Significant changes ($P < 0.01$) occurred in parr coloration between all age groups except between age-groups 5 and 6 (Duncan's multiple range test). Fish from age-group 5 could clearly be identified as smolts, as they had little parr coloration. Significant changes in condition factor ($P < 0.01$) occurred between all age groups except between 2 and 3 (Duncan's multiple range test).

Experiment 1: Olfactory Learning during Smoltification

Resting heart rate (beats per minute) increased continuously from age-group 1 (70.5 beats/min) to age-group 6 (81.4 beats/min) (further details in Morin et al. 1989). This could have influenced subsequent cardiac deceleration in the context of cardiac conditioning to L-cysteine. However, since there were no significant correlations between initial heart rate during

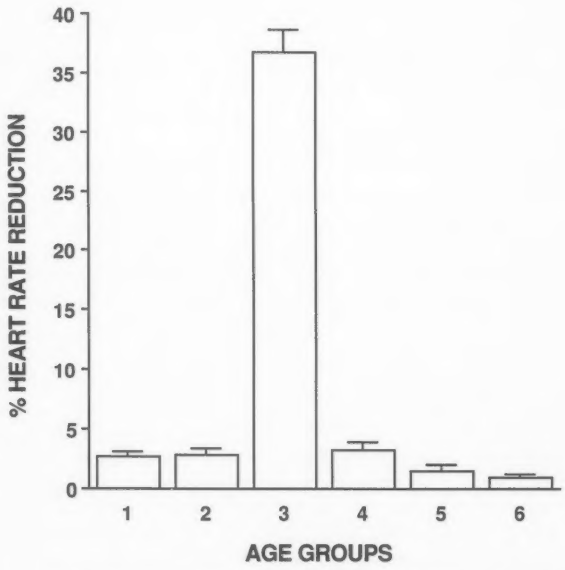

FIG. 4. Mean percentage of heart rate reduction in 2-yr-old Atlantic salmon exposed to L-cysteine (3.8×10^{-4} M) during a 10-trial session. Six age groups of 10 fish had been preexposed to L-cysteine (10^{-4} M) during an 8-d interval of the smoltification period (age-groups 1–6: first to sixth age group from the beginning of smoltification) and were tested at a fixed-time interval after the end of an initial odor exposure. In each age group, testing began 55 d after the end of odor exposure. The vertical bars represent the mean ± SE.

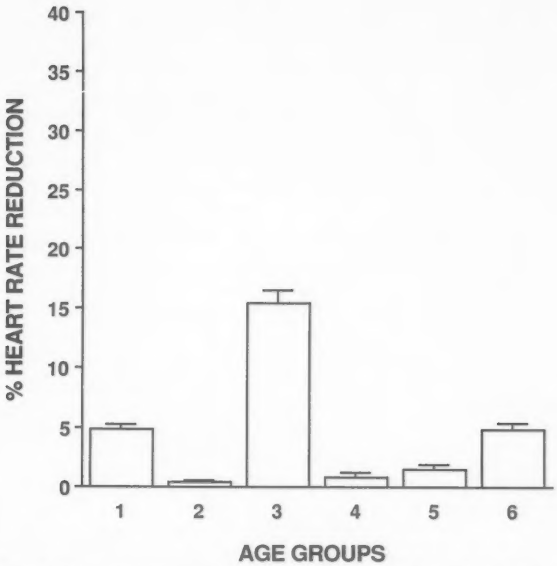

FIG. 5. Mean percentage of heart rate reduction in 2-yr-old Atlantic salmon exposed to L-cysteine (3.8×10^{-4} M) during a 10-trial session. Six age groups of 10 fish had been preexposed to L-cysteine (10^{-4} M) during an 8-d interval of the smoltification period (age-groups 1–6: first to sixth age-group from the beginning of smoltification) and were tested at the same age following initial odor exposure. In age-groups 1–6, testing began 85, 75, 65, 55, 45, and 35 d, respectively, after the end of odor exposure. The vertical bars represent the mean ± SE.

acclimatization and subsequent heart rate reductions at each block of trials (ranging from $r = 0.02$ to 0.22; $P > 0.05$), it was not necessary to conduct repeated measures analysis of covariance (Pedhazur 1982).

A three-way multiple analysis of variance (age groups × treatment groups × blocks of trials) with repeated measures on blocks of trials was used to reveal main effects on cardiac response to L-cysteine (Fig. 3) (Harris 1975; SPSS-X 2.1). Blocks of trials differed from each other ($F = 62.29$; df = 7,102; $P < 0.01$) but interaction effects were also found for age groups × blocks ($F = 1.76$; df = 35,431; $P < 0.01$), treatment groups × blocks ($F = 38.87$; df = 7,102; $P < 0.01$), and for age groups × treatment groups × blocks ($F = 2.16$; df = 35,431; $P < 0.01$). A univariate analysis of variance was used for analyzing simple main effects of age groups and treatment groups at different blocks of trials. At blocks 6, 7, and 8, heart rate reduction in conditioned fish of all age groups was greater than that of fish tested under a random-pairing situation (F values varied from 9.20 to 96.08 and were all significant at $P < 0.01$).

Another way to decompose interaction effects was to perform an analysis of simple effects between age groups within each treatment group. It showed overall differences in heart rate reduction to L-cysteine between age groups of conditioned fish ($F = 5.37$; df = 5,108; $P < 0.01$) but none between age groups of control fish tested under the random pairing situation. Since most conditioned fish (age-groups 1, 2, 3, 4, and 6) reached their best performance at the end of the second experimental session, we examined the relative importance of their heart rate reduction at block 8 by comparing the age groups of smoltification. Overall differences were found at block 8 ($F = 8.31$, df = 5,108; $P < 0.01$) and the Duncan's multiple range test revealed greater heart rate reduction in conditioned fish from age-groups 3 and 6 than in conditioned fish from any other age group of smoltification ($P < 0.05$). Heart rate reduction for age-group 3 (34.8%) was similar to that of age-group 6 (32.8%) whereas heart rate reduction for age-groups 1 (22.8%), 2 (21.6%), 4 (22.3%), and 5 (18.2%) did not differ.

Experiments 2 and 3: Odor Preexposure and Recognition

When tested for odor recognition, some fish that had been exposed to L-cysteine during smoltification reduced their heart rate in the presence of the same odorant. Overall differences in heart rate responses to L-cysteine were found between fish of different age groups of smoltification ($F = 63.41$; df = 5,54; $P < 0.01$) when they were all tested 55 d after the end of their initial odor exposure (Fig. 4). Cardiac deceleration reached 37% in fish from age-group 3, and according to a Duncan's multiple range test, this value was higher than that of any other age group of smoltification ($P < 0.01$). Heart rate reduction was similar in age-groups 1, 2, 4, 5, and 6 and did not exceed 5%. Significant differences were found in fish from different age groups of smoltification ($F = 19.93$; df = 5,54; $P < 0.01$) when they were tested at a different time interval following odor exposure (Fig. 5). When tested 65 d after the end of initial odor exposure, heart rate reduction to L-cysteine reached 15% in age-group 3 and this performance was more pronounced than that of any other age group of smoltification ($P < 0.01$) in which the interval between exposure and testing was longer (age-group 1: 85 d; age-group 2: 75 d) or shorter (age-group 4: 55 d; age-group 5: 45 d; age-group 6: 35 d). Cardiac deceleration in age-groups 1, 2, 4, 5, and 6 did not differ and never exceeded 5%.

Discussion

Imprinting is characterized by two interdependent events: (1) a greater ability for learning discrete environmental cues during a relatively short time interval and (2) a capacity to retain this information in long-term memory to fulfill a biological function. When both characteristics are present, the short interval during which learning ability and the capacity for long-term memory is optimal is called the sensitive period for imprinting. They remain valuable criteria of imprinting despite a considerable broadening of the original concept (Immelmann and Suomi 1981). The following discussion specifically addresses the question: are the aforementioned criteria of imprinting distinguishable in the present series of experiments designed to verify the existence of olfactory imprinting in Atlantic salmon?

The first criterion of imprinting refers to a heightened learning ability during a relatively short time interval. This was achieved in fish from age-groups 3 and 6 conditioned to L-cysteine during smoltification. A greater ability for olfactory learning prevailed in these fish as compared with any other age group of smoltification, as they exhibited significantly greater heart rate reductions to L-cysteine.

The second criterion of imprinting deals with a long-term retention of the information learned during a short time interval. Smolts previously exposed to L-cysteine as age-group 3 reduced heart rate to L-cysteine significantly more than smolts preexposed to L-cysteine during any other interval of smoltification. These fish were able to recognize the odor of their preexposure experience, and we conclude that fish of age-group 3 reached

the second criterion of imprinting. This recognition ability persisted irrespective of the length of time from the end of odor exposure to testing (Experiment 2) or the age of testing (Experiment 3). However, heart rate reduction to L-cysteine in smolts preexposed during age-group 3 of smoltification decreased when measured 65 d (Experiment 3) as compared with 55 d (Experiment 2) after the end of odor exposure. Because subjects remained in freshwater after smoltification and for several months prior to testing, we propose that the weakening of the cardiac response may have been due to the process of desmoltification (Evropeytseva 1963). A reappearance of parr characteristics (e.g. contrasting dark back and yellowish belly) was observed in fish tested 65 d after the end of odor exposure.

Both criteria of imprinting, a greater ability for learning during a relatively short time interval and a long-term memory of the information learned during this interval, were present only in fish from age-group 3 of smoltification. Clearly, the existence of a SPOI was demonstrated in these fish. Fish from age-group 6 were less able to recognize their preexposed odor, since heart rate reduction in these fish was relatively small. Thus, contrary to fish from age-group 3, fish from age-group 6 failed to reach the second criterion of imprinting.

Our results indicate that there is only one SPOI during smoltification (age-group 3) even though there appears to be two distinct optimal periods for olfactory learning (age-groups 3 and 6). What could have caused a greater ability for olfactory learning in fish from age-group 6 if it was not associated with an imprinting process as in fish from age-group 3? Morin et al. (1989) proposed that thyroid hormones facilitate olfactory learning during smoltification and found significant positive correlations between histological indexes of thyroid activity and heart rate conditioning to L-cysteine during smoltification. Thyroid activity as indicated by histological indexes was greatest in fish from age-groups 3 and 6. Morin et al. (1989) also proposed that thyroid hormones facilitate olfactory imprinting. As evidence, they reported apparently different plasma–tissue fluxes of thyroxine between age-groups 3 and 6, suggesting the existence of a thyroid–brain mechanism for olfactory imprinting in fish from age-group 3 but not in fish from age-group 6. Thus, thyroid hormones seem to exert a general facilitative effect on olfactory learning (age-groups 3 and 6) and a more specific one on olfactory imprinting (age-group 3).

Although we have demonstrated a SPOI in Atlantic salmon, this must not be taken to imply that olfactory imprinting is an all-or-nothing affair. In accordance with current views of imprinting, we have used the expression "sensitive period" without the restrictive connotation of the expression "critical period." During a sensitive period for imprinting, the animal's capacity to learn and store information in memory is optimal, implying that some of this capacity may persist beyond the sensitive period. Conversely, none of these capacities for learning and memory would occur beyond a "critical period" for imprinting. The concept of a critical period for imprinting appears untenable. In birds, the irreversibility of filial imprinting (i.e. an incapacity to learn beyond the critical period) and its influence on later sexual preferences are controversial issues (Bateson 1966). Beyond the so-called critical period, birds can still develop social attachments providing they are sufficiently familiar with the imprinted object (Hoffman 1987). In the present study, some imprinting may have occurred in more than one age group of smoltification. Conditioned fish from all six age groups of smoltification reduced their heart rate to L-cysteine suggesting that learning ability was not unique to fish from age-group 3 or 6. In addition, all smolts preexposed during smoltification reduced to some extent their heart rate in the presence of L-cysteine. For example, heart rate reduction to L-cysteine reached 3% (Experiment 2) or 5% (Experiment 3) in fish from age-group 1. In other experiments (unpubl. data), we have shown that unconditioned heart rate reduction to L-cysteine reached 0.64% (± 0.44 SD) in fish with no prior exposure to L-cysteine and tested at the same time as fish from age-group 3 (Experiment 2). Thus, some imprinting may have occurred beyond the optimal SPOI even though olfactory imprinting was clearly most pronounced in fish from age-group 3.

It could be argued that greater heart rate reductions in some fish conditioned to L-cysteine during smoltification were due to an increase in the general reactivity of fish to environmental cues instead of a heightened ability for olfactory learning. However, the magnitude of heart rate reductions did not change between trials or between different age groups of smoltification in fish trained under a random presentation of the odor–shock sequence. This suggests no change in the reactivity of fish to L-cysteine, during the course of the study. In addition, a greater learning ability during smoltification did not occur because of a greater reactivity to the shock as its intensity was adjusted, at the beginning of each training session, to induce bradycardia identical for every fish used in the experiment.

Although not measured in the present study, olfactory sensitivity may increase concomitantly with an olfactory learning ability during smoltification to facilitate olfactory imprinting in anadromous salmonids. The possibility that greater olfactory sensitivity during smoltification plays a role in the imprinting process was investigated by Rehnberg et al. (1985). In their study, the olfactory sensitivity of juvenile cohos was assessed by measuring avoidance responses to an amino acid (L-serine or L,D-alanine) presented before, during, and after smoltification. Threshold concentrations were lower before (10^{-7} M) than during smoltification (10^{-5} or 10^{-6} M). These results demonstrated no increase in sensitivity during smoltification and apparently indicate that olfactory sensitivity plays no role in the imprinting process. However, the possibility of heightened olfactory sensitivity during smoltification cannot be rejected, as Rehnberg et al. (1985) did not specify exactly when fish were tested during smoltification and apparently pooled data obtained throughout the period of smoltification. Under such circumstances, a brief period of heightened olfactory sensitivity would have been difficult to detect.

In the present study, we used an autonomic response (heart rate) instead of motor responses (following behavior) to identify an imprinting process. In juvenile salmon, unconditioned heart rate reduction to an odor previously experienced during smoltification may be regarded as an orientation response to an imprinted stimulus. In classical examples of filial imprinting, orienting movements of newly hatched birds (e.g. stretching of the neck) towards a previously experienced decoy are generally associated with a preference for that imprinted stimulus or a tendency to follow it. For this reason, orienting responses are reliable measures of the imprinting process in animal studies (Hess 1973).

In other fish studies, the expression "arousal response" is used to describe an unconditioned cardiac deceleration to a novel stimulus (Laming and Savage 1980; Laming and Ennis 1982; Rooney and Laming 1988). In contrast, we use the expression "orientation response" to designate an unconditioned cardiac deceleration elicited by a familiar stimulus. In the present study, we have shown in smolts that an uncondi-

tioned cardiac deceleration occurs in the presence of a previously experienced odor, L-cysteine (3.8×10^{-4} M), because of its recognition in the context of olfactory imprinting. Smolts of the same age do not decelerate heart rate to L-cysteine at a concentration of 3.8×10^{-4} M if they have not been preexposed to that stimulus (unpubl. data).

Our results suggest that olfactory imprinting in anadromous salmonids is controlled by two different mechanisms which seem to interact with each other in a specific manner. The first mechanism may act to increase the general capacity of fish for learning olfactory cues. Thyroid hormones may facilitate olfactory learning by increasing the excitability of fishes' nervous system during smoltification (Morin et al. 1989). Such learning capacity, however, may not in itself be sufficient for olfactory imprinting to occur. Accordingly, long-term olfactory memory was not demonstrated in one of our high-learning age groups of smoltification (age-group 6). Thus, a second mechanism may be involved in the consolidation of the odor information into long-term memory.

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Thyroid Activity Concomitant with Olfactory Learning and Heart Rate Changes in Atlantic Salmon, *Salmo salar*, during Smoltification[1]

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Thyroid-histological (epithelial cell height, follicular eccentricity) and thyroid-radiochemical (thyroxine, triiodothyronine) activities were examined in Atlantic salmon, *Salmo salar*, undergoing smoltification in relation to cardiac conditioning to L-cysteine (olfactory learning). Changes in plasma levels of thyroxine were also examined along with those of resting heart rate, during and after smoltification. In a related study, we reported greater learning ability in age-groups 3 (612–619 d since birth) and 6 (642–649 d) as well as a greater long-term olfactory memory in age-group 3. In the present study, thyroid-histological activity was correlated with olfactory learning during smoltification. Higher histological values occurred concomitantly with greater learning in age-groups 3 and 6. During smoltification, changes in thyroid-histological activity were different from those of radiochemical activity. Apparently different plasma–tissue fluxes of thyroid hormones occurred between age-groups 3 and 6. Plasma thyroxine was correlated with resting heart rate. Our results suggested that thyroid hormones play a role in olfactory learning and imprinting in anadromous salmonids.

Les caractéristiques histologiques (hauteur des cellules épithéliales, excentricité folliculaire) et radiochimiques (thyroxine, triiodothyronine) thyroidiennes sont examinées chez des saumons atlantiques, *Salmo salar*, au cours de la "smoltification" en relation avec le conditionnement d'une réponse cardiaque à la L-cystéine (apprentissage olfactif). Les changements des niveaux plasmatiques de thyroxine sont également évalués avec ceux de la fréquence cardiaque au repos, pendant et après la smoltification. Parallèlement, nous avons décelé une plus grande habileté à l'apprentissage olfactif chez les groupes d'âge 3 (612–619 d depuis la naissance) et 6 (642–649 d) ainsi qu'une meilleure mémoire olfactive à long terme chez le groupe d'âge 3. Dans le cadre de la présente étude, l'activité histologique thyroidienne est corrélée avec l'apprentissage olfactif pendant la smoltification. Des valeurs histologiques plus élevées sont apparues concomitamment à un plus grand apprentissage olfactif chez les groupes d'âge 3 et 6. Au cours de la smoltification, les changements dans l'activité histologique thyroidienne étaient différents de ceux de l'activité radiochimique. Des flux plasma-tissulaires apparemment différents d'hormones thyroidiennes se sont produits entre les groupes d'âge 3 et 6. La thyroxine plasmatique était corrélée avec la fréquence cardiaque au repos. Nos résultats suggèrent que les hormones thyroidiennes jouent un rôle dans l'apprentissage et dans l'empreinte olfactive chez les salmonidés anadromes.

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In salmonids, an active thyroid gland at the time of smoltification (Hoar 1939; Dickhoff et al. 1978) or during seaward migration (Hoar and Bell 1950; Hoar 1951) has been associated with hyperactivity (Fontaine et al. 1952; Leloup and Fontaine 1960) and also with a heightened sensitivity (or excitability) to external stimuli (Hoar 1953; Baggerman 1960; Saunders and Henderson 1970; Fontaine 1975). Salmonid juveniles injected with thyroid hormones become more active (Hoar et al. 1952; Hoar et al. 1955), more sensitive to odors (reviewed by Gorbman 1969), and better in learning ability (Lindberg and Scholz, cited in Scholz 1980). A neuronal basis for these effects is indicated by the recent findings of an intranuclear triiodothyronine (T3) binding site in the brain of salmonids exhibiting several characteristics of mammalian T3 receptors (Scholz et al. 1985; Bres and Eales 1988). Besides regulating the morphological, physiological, and behavioral changes of the parr–smolt transition (reviewed by Hoar 1976), these observations suggest that thyroid hormones affect the central nervous system of salmonid juveniles during smoltification.

The influence of thyroid hormones on growth, development, and normal functioning of the central nervous system is best known in higher vertebrates (Ford and Cramer 1977; Nunez 1984). There is a critical period in neonatal mammals during which thyroid hormones play an essential role in differentiating brain-cell structures (Hamburgh 1969). Hypothyroidism during this period causes permanent alterations of normal brain functioning characterized by behavioral defects including learning

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impairments (Meisami 1983; Tamasy et al. 1986; Rial et al. 1987). Relatively high concentrations of brain nuclear T3 receptors (Oppenheimer et al. 1974; Gullo et al. 1987; Ferreiro et al. 1988) have been identified, suggesting the existence of a thyroid–brain mechanism regulating the functioning of the nervous system during development.

The precision of homing behavior in anadromous salmonids has been attributed to the existence of a sensitive period for olfactory imprinting occurring during smoltification (reviewed by Hasler and Scholz 1983). Scholz (1980) hypothesized that high levels of thyroid hormones facilitate olfactory imprinting in coho salmon, *Oncorhynchus kisutch*, undergoing smoltification. Hasler and Scholz (1983) reported an experiment in which presmolt cohos injected either with gonadotropin (ACTH) to induce a migratory state or with a saline solvent (SS) were divided in two subgroups and further injected with the thyroid stimulating hormone (TSH) or with SS concomitantly with exposure to an artificial odorous chemical (morpholine or phenethyl alcohol). All fish were subsequently tested in a stream scented with the artificial odorous chemical. The results of this experiment showed that fish treated with ACTH + TSH as compared with those injected differently (ACTH + SS, SS + TSH, or SS + SS) were attracted more reliably to their imprinted odor. Heart rate responses to imprinting odors were also examined in these fish. Again, compared with any other group of injected fish, those treated with ACTH + TSH reacted significantly more to the imprinted odor by an unconditioned cardiac deceleration, as when they were stimulated with a nonfamiliar chemical. Hasler and Scholz (1983) concluded that TSH injections activated olfactory imprinting in smolts. Through their effect on the central nervous system, thyroid hormones may influence the imprinting process. Newly hatched birds imprint until a few hours after birth (Hess 1973) during which high levels of thyroid hormones are found in their blood. (Thommes et al. 1977). Delayed maturation of the central nervous system of prehatched birds by thyroid-blocking agents (Chandrasekhar et al. 1979) may interfere with the imprinting process.

A sensitive period for olfactory imprinting (SPOI) has been identified in Atlantic salmon, *Salmo salar*, undergoing smoltification (Morin et al. 1989). The magnitude of a conditioned cardiac response to L-cysteine was measured in fish during six 8-d intervals within the parr–smolt transition. We postulated that odor learning would be optimal during a sensitive period for imprinting and that the magnitude of a conditioned cardiac deceleration would significantly increase at such times. In addition, olfactory memory was assessed by exposing fish to L-cysteine during the same six 8-d intervals of smoltification and by measuring their unconditioned cardiac deceleration to L-cysteine after smoltification. We postulated that the magnitude of cardiac deceleration to L-cysteine would be significantly greater among fish exposed to L-cysteine during a sensitive period for imprinting. Greater conditioned cardiac decelerations to L-cysteine occurred in age-groups 3 and 6 as compared with any other age group of smoltification. Fish tested for odor recognition exhibited a greater unconditioned cardiac deceleration to L-cysteine if they had been exposed during the third age interval of smoltification. Morin et al. (1989) concluded that there are two distinct optimal intervals for olfactory learning (age-groups 3 and 6) during smoltification but that there is only one "sensitive" period for olfactory imprinting (age-group 3), occurring between 21 and 28 d after the onset of smoltification induced in the laboratory.

The principal objective of the present study was to determine in Atlantic salmon if a relationship exists between thyroid hormones, olfactory learning, and olfactory imprinting. We proposed that higher thyroid activity significantly correlated with greater ability for heart rate conditioning to L-cysteine during smoltification would be evidence of the role of thyroid hormones in olfactory learning (age-groups 3 and 6) or in olfactory imprinting (age-group 3).

To assess thyroid activity during smoltification, we used two histological measures, epithelial cell height and follicular eccentricity, and two radiochemical measures, plasma levels of thyroxine (T4) and T3. Epithelial cell height generally increases with plasma levels of thyroid hormones during smoltification, indicating a heightened thyroid activity (Eales 1963, 1965; Nagahama et al. 1982). When thyroid activity is least, follicular cells are generally flattened, but when greater, the cells are cuboid (Hibiya 1982) or columnar (Barrington 1975; Bloom and Fawcett 1975). Infolding and tufting follicles have also been identified in very active glands of fish during smoltification (Hoar 1939; Fontaine et al. 1952), suggesting that the shape of thyroid follicles changes with thyroid activity. Since we found, in a preliminary study, that the tendency of circular follicles to become elliptic increases with cell height during smoltification, we quantified this tendency with a measure of "follicular eccentricity" as an additional and novel index of thyroid activity during smoltification.

Another objective was to assess the relationship between resting heart rate and plasma levels of T4 during and after smoltification. Thyroid hormones are known to increase heart rate, presumably by affecting the excitability of the nervous system (Guyton 1986). Injections of small quantities of T4 in the blood of rainbow trout, *Salmo gairdneri*, increase heart rate and the excitability of the nervous system to chemical stimuli (Oshima et al. 1972). We proposed that high levels of T4 significantly correlated with high values of resting heart rate during smoltification would be evidence of the role of thyroid hormones on the excitability of fishes' nervous system.

Materials and Methods

Fish and Overall Procedure during and after Smoltification

The two hundred and forty 2-yr-old Atlantic salmon were those described in Morin et al. (1989). One hundred and twenty fish had been trained during smoltification for heart rate conditioning to L-cysteine or with a random odor–shock presentation (Experiment 1, Morin et al. 1989) whereas another 120 fish had been preexposed to L-cysteine during smoltification and tested for odor recognition after smoltification (Experiments 2 and 3, Morin et al. 1989). In the present study, we assessed (1) thyroid-histological and thyroid-radiochemical activities in fish conditioned to L-cysteine during smoltification and (2) plasma levels of T4 and resting heart rate in fish conditioned to L-cysteine or trained with a random odor–shock presentation during smoltification and in those preexposed to L-cysteine during smoltification and tested for odor recognition after smoltification. The age since birth of conditioned fish from each of the six age groups of smoltification (age-groups 1–6) was as follows: age-group 1: 592–599 d, age-group 2: 602–609 d, age-group 3: 612–619 d, age-group 4: 622–629 d, age-group 5: 632–639 d, age-group 6: 642–649 d.

When tested for odor recognition during the postsmoltification period, the age of each group of fish (age-

groups 7–12) preexposed during six equal intervals within smoltification (age-groups 1–6) was as follows: age-group 7: 654 d, age-group 8: 664 d, age-group 9: 674 d, age-group 10: 684 d, age-group 11: 694 d, age-group 12: 704 d.

We used an additional 16 fish in this study (see age-group 11 in Fig. 1) which had already been trained for heart rate conditioning to L-cysteine in one of our pilot studies. All other details concerning subjects, apparatus, and the procedure used for cardiac conditioning to L-cysteine are found in Morin et al. (1989).

Thyroid Histology

Fish were sacrificed at times between 1000 and 1300 the day after conditioning. Sampling to collect thyroid tissues occurred at 2-d intervals between 1 January and 26 February 1986. Glandular histology was performed on each fish used for Experiment 1 (Morin et al. 1989). Following plasma sampling (see below), the subpharyngeal tissue containing the thyroid gland was dissected out and fixed in a Bouin solution for light microscopy. Each piece of tissue was embedded in paraffin and 5- to 6-μm-thick serial sections were made. Samples were stained with hematoxylin–eosin or with periodic acid shift. Thyroid structures were magnified, photographed, and measured on an electronic plotting table connected to an IBM microcomputer.

Thyroid activity was assessed by measuring the height of follicular epithelial cells and follicular eccentricity (i.e. deviation from an elliptic shape). All follicles in a given fish were measured and an average size was estimated. For each fish, five follicles measuring within 1 standard deviation of the average follicle size were randomly selected. They were measured for their eccentricity and the thickness of their epithelial lining (epithelial cell height). Eccentricity (E) was derived from the following calculation:

$$E = \sqrt{1 - \left(\frac{a}{b}\right)^2}$$

where a = smallest diameter and b = largest diameter.

Plasma Collection and Thyroid Hormone Determination

Sampling to collect plasma occurred at 2-d intervals between 1 January and 26 February 1986 and at 10-d intervals between 3 March and 22 April of the same year. After fish had been lightly anesthetized and assessed for body coloration, the tail (wiped dry) was severed and blood was aspirated from the caudal section of the dorsal aorta into heparinized tuberculin syringes. The blood was transferred to microhematocrit capillary pipettes treated with heparin for centrifugation. Upon separation from blood, the plasma was stored frozen at −20°C in 0.3-mL plastic microcentrifuge tubes until assayed for T4 and T3.

The quantity of blood taken from each fish was sufficient for T4 determination but not for T3. Consequently, a T3-radioimmunoassay was performed for each age group of smoltification on a single "pooled" sample containing the blood of all 20 fish trained in the presence of L-cysteine (see Experiment 1 in Morin et al. 1989). Hormone determinations on pooled samples (T3) or on individual samples (T4) were made with radioimmunoassay test kits (T4: Becton Dickinson Co.; T3: Kallestad Laboratories, Inc.). Since these tests are clinically used for human thyroid hormones, aliquots of the standards had to be made for plotting a standard curve fit to identify small quantities of hormones typically found in salmon plasma. Standards and samples were run in duplicate. Calculation of radioimmunoassay results was made by a LKB-Wallac Rack-gamma counter utilizing the spline-function program.

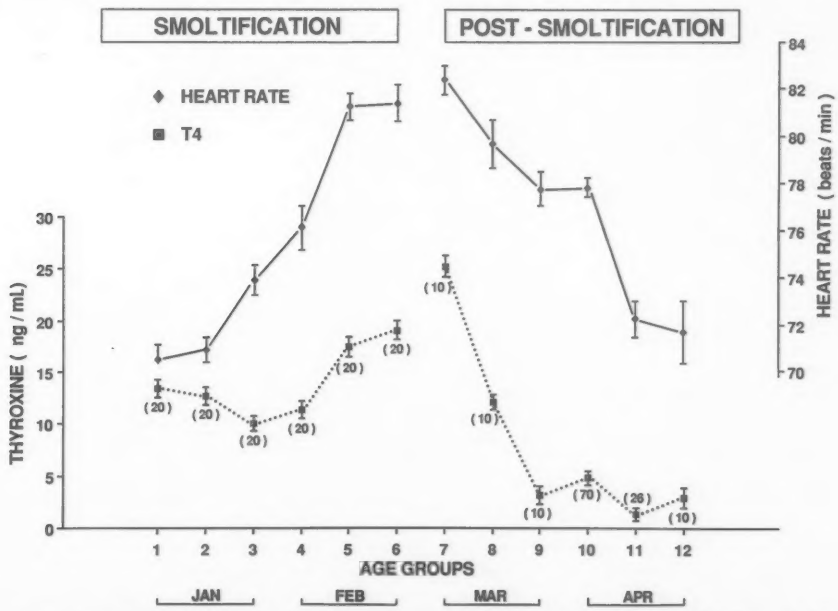

FIG. 1. Physiological changes in 2-yr-old Atlantic salmon between the beginning of January and the end of April 1986. Heart rate and plasma thyroxine (T4) levels were measured in six age groups of smoltification and six age groups of postsmoltification. Size of samples is shown in parentheses and the vertical bars represent the mean ± SE.

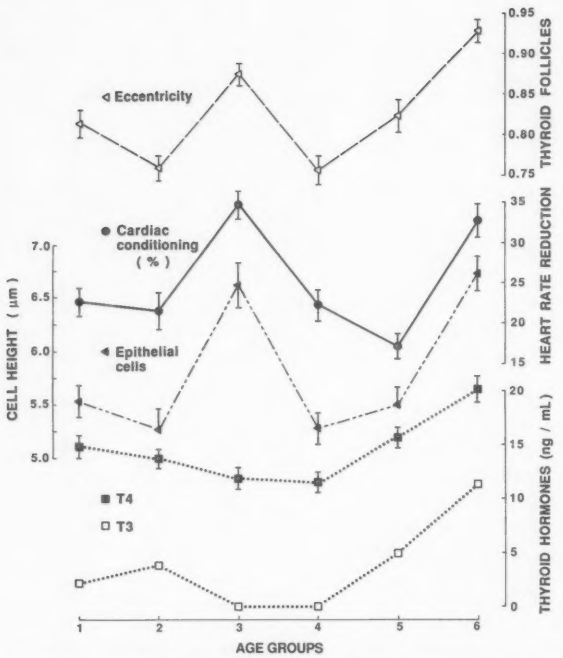

FIG. 2. Relationships between heart rate conditioning to L-cysteine and thyroid activity during smoltification, in 2-yr-old Atlantic salmon. Cardiac conditioning was measured at the end of the second testing session (day 2). Plasma levels of thyroxine (T4) and triiodothyronine (T3), epithelial cell height, and follicular eccentricity measurements were derived from samples collected on day 3. An eccentricity score of 1 is indicative of a perfect ellipse, while a score of 0 describes a circle. The vertical bars represent the mean ± SE.

Results

T4 and Resting Heart Rate during and after Smoltification

From a simple regression analysis, the pattern of variation in resting heart rate from age-group 1 to 12 was similar to that of plasma levels of T4 ($F = 17.06$; df = 1,118; $P < 0.01$; Fig. 1). Consistent differences occurred from age-group 1 to 12 in fish's heart rate ($F = 7.85$; df = 11,244; $P < 0.01$; one-way ANOVA) as well as in plasma levels of T4 ($F = 59.39$; df = 11,244; $P < 0.01$). Heart rate increased rapidly from age-group 1 to 5, stabilized at age-group 6 and 7, and then decreased regularly until the end of the study (age-group 12). Levels of T4 remained unchanged from age-group 1 to 4 but then increased regularly to reach a maximum of 25.2 ng/mL for age-group 7. A drastic reduction in T4 followed for age-group 8, and from age-group 9 to 12, small quantities of T4 remained in fishes' blood.

Olfactory Learning and Thyroid Activity during Smoltification

Figure 2 depicts thyroid activity and heart rate reduction in different age groups of 10 fish conditioned to L-cysteine during smoltification. Data from fish trained under a random pairing situation (see Morin et al. 1989) are not included here. It is worth mentioning, however, that for each index of thyroid activity analyzed separately (follicular eccentricity, epithelial cell height, or plasma levels of T4), there was no difference between the values obtained in conditioned fish and those obtained in the random pairing group (t-tests). This should be kept in mind considering that each mean value of plasma T4 shown in Fig. 1 and calculated from 20 fish (conditioning and random pairing groups) looks somewhat different from that shown in Fig. 2, calculated from only 10 conditioned fish.

From simple regression analyses, changes in the magnitude of cardiac conditioning, across the different age groups of smoltification, were similar to those of epithelial cell height ($F = 5.60$; df = 1,58; $P < 0.05$) and of follicular eccentricity ($F = 4.07$; df = 1,58; $P < 0.05$). In addition, changes in epithelial cell height were similar to those of follicular eccentricity ($F = 4.28$; df = 1,58; $P < 0.05$). Changes in plasma T3 were not analyzed statistically due to sample size. Nevertheless, from age-group 2 to 6, changes in plasma T3 resembled those of plasma T4. Plasma T4 was not correlated with cardiac conditioning, epithelial cell height, or follicular eccentricity.

Overall differences between the age groups of smoltification were found for cardiac conditioning ($F = 4.98$; df = 5,54; $P < 0.01$; one-way ANOVA), epithelial cell height ($F = 4.79$; df = 5,54; $P < 0.01$), and follicular eccentricity ($F = 6.45$; df = 5,54; $P < 0.01$). For cardiac conditioning, cell height, and follicular eccentricity, significant differences ($P < 0.01$) between successive age groups existed between age-groups 2 and 3, 3 and 4, as well as between age-groups 5 and 6 (Duncan's multiple range test). From age-group 1 to 6, overall differences in plasma T4 levels were found in conditioned fish ($F = 6.02$; df = 5,54; $P < 0.01$). Plasma T4 remained unchanged from age-group 1 to 4 and then increased significantly from age-group 4–5 and from age-group 5–6 ($P < 0.05$).

Discussion

Plasma levels of T4 increased during smoltification to subsequently decline during the postsmoltification period. This pattern of T4 fluctuations is consistent with previous studies on Atlantic salmon undergoing smoltification under natural (Lindahl et al. 1983; Boeuf and Prunet 1985; Dickhoff et al. 1985; Virtanen and Soivio 1985; McCormick et al. 1987) or long but constant photoperiods (Virtanen and Soivio 1985; McCormick et al. 1987). Despite the high functional state of the thyroid in fish from age-group 3, it can be argued that thyroid activity was relatively low in those fish, since the "surge" of plasma T4 was most apparent near the end (age-groups 5 and 6) and soon after smoltification (age-group 7). However, compared with recent studies on Atlantic salmon reporting concentrations of plasma T4 of about 10 ng/mL at the onset of smoltification (Boeuf and Prunet 1985; Dickhoff et al. 1985; Virtanen and Soivio 1985; McCormick et al. 1987), our initial value of T4 measured in fish from age-group 1 of smoltification was relatively high, as it reached about 14 ng/mL.

A key issue of this research was to show significant positive correlations between thyroid-histological activity and olfactory learning in Atlantic salmon undergoing smoltification. More specifically, we have shown that a greater ability for cardiac conditioning to L-cysteine, as in fish from age-groups 3 and 6, was associated with higher values of epithelial cell height and follicular eccentricity. This suggests that thyroid hormones facilitate olfactory learning during specific intervals within smoltification. Thyroid hormones may facilitate olfactory learning by affecting the excitability of fishes' nervous system. In higher vertebrates, these hormones are known to affect excitability by increasing the activity of adrenergic receptors (Had-

ley 1988). As in some other teleosts, the heart of salmonids possesses adrenergic receptors (Laurent et al. 1983) whose sensitivity may be altered by thyroid hormones. Our results showing a significant correlation between plasma levels of T4 and resting heart rate indicate that thyroid hormones influence heart rate by affecting the excitability of fishes' nervous system. This is consistent with the observations of Oshima et al. (1972) who reported lower heart rate and lower excitability to chemical stimuli (EEG recordings) in thyroidectomized as compared with normal rainbow trout. They also reported higher heart rate and higher excitability to chemical stimuli in thyroidectomized as well as in normal fish injected with a low dosage of T4.

A discrepancy between histological and radiochemical indexes of thyroid activity existed in fish from age-group 3 but not in fish from age-group 6. In fish from age-group 3, a significant increase in epithelial cell height and follicular eccentricity occurred in the absence of any change in plasma T4. In contrast, a significant increase in cell height and follicular eccentricity occurred in fish from age-group 6 concomitantly with a significant increase in plasma T4. Such differences in T4 kinetics between fish from age-groups 3 and 6 coincide with contrastingly different phenomena: olfactory learning (age-groups 3 and 6) and olfactory imprinting (age-group 3). Morin et al. (1989) reported a significantly greater ability for olfactory learning in fish from age-groups 3 and 6 but a significantly greater capacity for long-term olfactory memory only in fish from age-group 3. These findings suggest that thyroid hormones may influence olfactory imprinting by exerting a specific thyroid–brain effect rather than a general effect on the fishes' nervous system. This hypothesis is consistent with the idea of a thyroid–brain mechanism activating olfactory imprinting in anadromous salmonids (Hasler and Scholz 1983; Scholz et al. 1985).

The discrepancy between plasma T4 and any of the thyroid-histological parameters, observed in smolts preexposed to L-cysteine as age-group 3, may be due to an incorporation of thyroid hormones by the brain and other target organs. We speculate that a rapid plasma–tissue flux of T4 occurred in fish from age-group 3 preventing a plasma T4 surge following heightened thyroid activity. Since morphological changes accompanying smoltification persisted in fish from age-group 3 (Morin et al. 1989), body tissues probably required great quantities of thyroid hormones to process these changes. This interpretation is supported by the findings of Specker et al. (1984) showing significantly greater quantities of T4 in tissues than in plasma of coho salmon during smoltification. At the onset of this developmental process, an efficient plasma–tissue flux of T4, lowering the level of T4 in fishes' blood, may indicate the involvement of slowly equilibrating tissues (Specker et al. 1984) including skin and brain as major targets of T4. Evidence that rapid incorporation of thyroid hormones by the brain (and other tissues) lowers plasmatic levels of these hormones was provided by Scholz et al. (1985) who found a progressive decline in blood T3 together with a gradual uptake of T3 in brain nuclei as well as in the whole brain tissue of rainbow trout injected with a tracer dose of radiolabelled T3.

Discrepancies between histological (i.e. cell height) and radiochemical indexes are reported elsewhere (Fontaine et al. 1953; Eales 1963, 1964, 1965; Drury and Eales 1968; Nishioka et al. 1985). Lack of correlation between these different indexes has been attributed to the heterogeneity of fishes' thyroid characterized by the coexistence within the same tissue of follicular units in different functional states (Eales 1979). Previous histological examinations have been unreliable (Sage and Robins 1970) and have based their measurements on individual epithelial cells from follicles selected at random on a given fish (Higgs et al. 1976; Milne and Leatherland 1980). To increase reliability in our assessment of thyroid activity, we took two instead of only one histological index. In addition, we measured the height of all epithelial cells in representative thyroid follicles. Furthermore, follicular eccentricity is a good sign of thyroid activity, as it was significantly correlated with epithelial cell height, a common criterion of thyroid activity. Higher follicular eccentricity in fish from age-group 3 or 6 could have been the result of follicle proliferation in highly active thyroids. This might have led follicles growing close to each other within the restricted confines of the basibranchial region to become mechanically distorted due to a "packing effect" (J. G. Eales, University of Manitoba, Winnipeg, Man., pers. comm.).

Greater olfactory learning reflecting an optimal capacity for encoding odor-related information during a smoltification-associated SPOI, together with long-term retention of odor information acquired during this sensitive period, may be attributed to the influence of thyroid hormones on brain maturation (Scholz and Hasler 1983; Scholz et al. 1985). Other suggestions have been made that imprinting occurs under high arousal (Kovach 1970; Martin and Schutz 1975) and high excitability (Bateson 1981) with important structural alterations in the central nervous system (Horn 1986). Internal changes involving hormones may also increase sensitivity to environmental stimuli at the time of imprinting in vertebrates (Landsberg and Weiss 1976; Weiss et al. 1977) including anadromous salmonids (Fontaine 1975).

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A Robust Method for Parameter Estimation from Catch and Effort Data

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The problem of robust estimation of optimal effort levels from surplus production models is considered. A variety of models are used to generate data, for the purpose of testing estimation schemes. The result of an estimation is an estimate of the optimal effort. These efforts are compared using the expected discounted value of a deterministic stock, which corresponds to the model used to generate the data. Such a criterion takes into account not only the loss due to bias in the estimated optimal effort, but also the loss due to the variance of the estimator. Estimation is difficult if there is a lack of informative variation in effort levels or stock sizes. In such cases, the estimation scheme which maximizes the criterion described above sacrifices realism in the representation of the stock–production relationship in order to reduce the variance of the estimate of optimal effort. We present a composite estimation scheme which performs acceptably in all the cases we have examined, and whose performance degrades slowly as the amount of information in the data decreases.

Le problème de l'estimation robuste des niveaux d'effort optimaux à partir de modèles de production excédentaire est analysé. Divers modèles sont utilisés pour fournir des données dans le but de tester les hypothèses d'estimation. Le résultat d'une estimation est une estimation de l'effort optimal. Ces efforts sont comparés à l'aide de la valeur actualisée prévue d'un stock déterministe, qui correspond au modèle utilisé pour produire les données. Un tel critère tient compte non seulement de la perte due au biais dans l'effort optimal estimatif, mais également de la perte due à la variance de l'estimateur. L'estimation est difficile lorsqu'il manque de variations informatives sur les niveaux d'effort ou la taille des stocks. Dans de tels cas, l'hypothèse d'estimation qui maximise le critère décrit ci-dessus sacrifie le réalisme de la représentation du rapport stock-production pour réduire la variance de l'estimation de l'effort optimal. Nous présentons ici un schéma d'estimation composite qui obtient une performance acceptable dans tous les cas que nous avons examinés et dont la performance diminue lentement à mesure que la quantité d'information des données diminue.

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Fisheries data are often not very informative about key parameters that describe responses to changing exploitation rates. The problem is not just that fisheries data are "noisy" because of inadequate sampling or effects of biophysical factors other than stock size and fishing effort. There is usually also a lack of informative variation in effort levels and stock sizes over time, so that estimation of optimum fishing policies involves extrapolation far beyond combinations of circumstances actually seen or "tested" in the history of the fishery. Data problems due to noise and lack of informative variation are often impossible or very expensive to remedy.

Can we devise estimation schemes which are able to cope with uninformative data? To answer this question it is important to have an appropriate criterion for effectiveness of an estimation scheme. We can certainly devise models that fit the data more precisely, by representing various sources of variation more realistically. But the literature of statistics warns that good model fits do not imply good prediction, or precise estimates of parameters (or combinations of parameters) which are of particular importance for policy. Perhaps the most accessible accounts of this warning are in discussions of optimal choice of explanatory variables in multiple regression schemes, but the principles apply quite generally. Draper and Smith (1981, p. 294–302) introduced the term "variance error" to describe the portion of the expected squared error which is due to variation (lack of precision) in the estimated parameters. For example, if an estimator z is normally distributed with mean 0 and variance σ^2, and if the loss associated with an inaccurate estimate is z^2, then the expected loss of employing z is σ^2. This quantity may be arbitrarily large, in spite of the fact that the estimator is unbiassed. Draper and Smith (1981) recommended use of a statistic such as C_p of Mallows (1973), which takes account of the variance error as well as the error due to bias. These ideas are developed at much greater length in Belsley et al. (1980) in discussing the problem of "collinearity" which frequently arises in multivariate data. They show how effective regression schemes will reduce the number of explanatory variables in order to reduce the variance error, even at the expense

of substantial lack of fit. Similar simplifications are commonly found to be optimal in time series predictions. A good introduction and review is given in Larimore and Mehra (1985). The criterion which they recommend is due to Akaike (1973, 1976). An additional reference is Söderström (1977).

Although these ideas cannot be carried over directly to fisheries problems because of the nonlinearity of the underlying models, it is clear that there is a trade-off between model accuracy (low bias error) and low sensitivity of the corresponding estimation scheme to such effects as fluctuations in the dynamics and errors in the data. In the case of fisheries data, we see an extreme example of such fluctuations and errors, coupled with low variation in stock size and effort levels. Thus we should expect that the most successful estimation schemes will tend to ignore niceties in the faithfulness of the representation of the underlying dynamics in order to improve the variance error of estimators of key policy determinants such as optimal fishing effort. Here we compare a number of models for stock and production and present a composite estimation scheme which is more robust in the above sense than any other known to us.

The literature on the analysis of catch and effort data contains many models for surplus production. Schnute (1985), following Deriso (1980), has classified and summarized these models, and embedded them in a single family containing a shape parameter. Schnute suggested that the choice between these models in any particular case may be made on the basis of the model identification methods of Box and Jenkins (1976). Here we will take a different approach, and will compare the performance of various estimation methods on simulated data (Ludwig et al. 1987). The success of each estimation scheme will be judged by the accuracy of the estimated optimal effort, and by the value of choosing such an effort for all future harvests. Value will be measured in terms of the discounted sum of future harvests. In each case, the distribution of this value function will be obtained from a set of 100 trials with random data. Such a criterion is analogous to the comparison of the expected squared error which is advocated by Draper and Smith (1981). In our earlier work, the Ricker and Power models for stock–production were compared. The power model assumes that production is proportional to a power of the stock size. It was shown that the Ricker model performed better than the Power model, even when the simulated data were generated using the Power model.

Here we extend that analysis to the generalized production functions which are described in Schnute (1985). The presentation is organized in three sections. A heuristic analysis of a simplified problem is carried out in Section 2. The results suggest that the family of generalized Ricker models given in (2.4b) below should be superior to those in the Deriso–Schnute family given in (2.4a), according to the criteria outlined above. Within the generalized Ricker family, no single model is superior to all the others, but we show that it is always better to estimate with a model which is slightly different from the model used in generating the data. These principles are verified in Section 3, by applying the various methods to simulated data. Our main result is that the correctness of the model as a representation of the underlying biological reality is less important than the statistical properties of the estimators. Moreover, within these simpler models, the Ricker model and its generalization appear to be as effective as any others, regardless of the underlying stock dynamics. Our analysis of the performance of the various models suggests a composite procedure, which appears to be robust. That is, it is not necessarily better than other methods in any particular situation, but its performance degrades slowly as the quality of the data deteriorates. The results of this paper and of Ludwig et al. (1987) are summarized in Section 4.

2. Analysis of a Simplified Problem

The nonlinear parameter estimation problem as described in Ludwig et al. (1987) is too complicated for analytical analysis. To understand the relative performance of the various stock–production models, we shall simplify the problem by assuming that the catchability q is known, and effective fishing efforts are observed without error. The resulting problem is linear in the case of the Ricker model and its generalization, which is introduced below. We can analyze these models and see that a proper choice of the generalized model can substantially reduce the sensitivity of the resulting estimator to process errors in the data. This improvement comes at the expense of a bias introduced by incorrect model structure. Finally, we give some arguments to indicate that the stock–production models in the Deriso–Schnute family may be expected to be inferior for estimation purposes.

In our simplified model, the catch C_t, observed effort E_t, and the stock biomass B_t are assumed to satisfy

$$(2.1)\quad C_t = B_t(1 - e^{-qE_t}).$$

The spawning stock S_t is given by

$$(2.2)\quad S_t = B_t - C_t = B_t e^{-qE_t}$$

and the following year's biomass is given by

$$(2.3)\quad B_{t+1} = f(S_t)e^{w_t}.$$

where the sequence $\{w_t\}$ is assumed to consist of independent normally distributed random variables with mean 0 and variance σ_w^2. Such a simple stock–production model ignores age structure in the population, but the results of Ludwig and Hilborn (1983) and Ludwig and Walters (1985) suggest that inclusion of age structure in the model decreases its effectiveness.

We shall be concerned with two specific forms for $f(S_t)$ below. Equations corresponding to these forms will be labelled with a and b respectively in the sequel. The stock–production model of Deriso and Schnute assumes that

$$(2.4a)\quad f(S_t) = S_t e^{\alpha}(1 - \beta\gamma S_t)^{1/\gamma}.$$

The case $\gamma = -1$ corresponds to the Beverton–Holt model, the limit as $\gamma \to 0$ corresponds to the Ricker model, and the case $\gamma = 1$ corresponds to the Schaefer model. In accordance with the principles explained in Draper and Smith (1981) and Belsely et al. (1980), one would not ordinarily attempt to estimate γ, because the resulting variance error in the estimates would produce a worse result than if a plausible value were assumed.

We shall define the generalized Ricker model as

$$(2.4b)\quad f(S_t) = S_t e^{\alpha - \beta S_t^p}.$$

For the same reason as above, the parameter p is usually chosen rather than estimated. If $p = 1$ and $\gamma \to 0$, the models (2.4a) and (2.4b) are identical.

We shall assess the performance of models (2.4a and 2.4b) by comparing the variance of the exploitation rate R which corresponds to the optimal stock size. Since q is assumed to be known, the determination of R is equivalent to the determina-

tion of the optimal effort. It is shown in Appendix B that in our simple case, R depends only upon $\hat{\alpha}$, and not upon $\hat{\beta}$. Thus to a linear approximation the variance of R is proportional to the variance of $\hat{\alpha}$. Consequently we shall study the dependence of the variance of $\hat{\alpha}$ upon the choice of model.

Choice of p in the Generalized Ricker Model

We may solve (2.3) and (2.4b) for w_t as follows:

$$(2.5b)\quad w_t = \log(B_{t+1}/f(S_t)) = Y_t - (\alpha - \beta X_t)$$

where

$$(2.6)\quad Y_t = \log(B_{t+1}) = \log(C_{t+1}/(1 - e^{-qE_{t+1}}))$$

and $X_t = S_t^p$. The method of least squares for (2.5b) is the same as a linear regression for α and β. The variance of $\hat{\beta}$ is given by

$$(2.7)\quad \sigma_{\hat{\beta}}^2 = \frac{\sigma_w^2}{s_X^2}$$

where

$$s_X^2 = \frac{1}{n} \sum_{i=1}^{n} (X_i^2 - \bar{X})^2$$

and the variance of $\hat{\alpha}$ is given by

$$(2.8)\quad \sigma_{\hat{\alpha}}^2 = \frac{\sigma_w^2}{n} + \bar{X}^2 \sigma_{\hat{\beta}}^2.$$

Equation (2.8) indicates that the variance of $\hat{\alpha}$ can be reduced increasing n or by reducing the second term in (2.8). It is shown in Appendix A that the latter term decreases as p increases. Therefore, one may expect improvement in the variance of $\hat{\alpha}$ by choosing very large values of p. However, such large values of p may produce unacceptable bias in the parameter estimates, due to lack of fit in the model. As was explained in the introduction, a proper criterion for the quality of an estimation scheme is the combination of error due to bias and that due to variance error. If one chooses the "correct" values of p, one minimizes the bias error in the estimate of R. Nearby values of R will show only slightly worse bias error, since the bias error is near a minimum. But these nearby values of p will show a linear relationship in the variance error, according to Appendix A. Therefore the minimum for the total error will be at a value of p somewhat higher than the "correct" one. The best choice of p will depend upon the particular data set in hand.

Choice between the Ricker and Deriso–Schnute Models

For the generalized Ricker model (2.4b) the regression of α and β is linear, as seen from (2.5b). On the other hand, for the generalized recruit models, the regression is nonlinear, since

$$(2.5a)\quad w_t = X_t - \left(\alpha + \frac{1}{\gamma} \log(1 - \beta\gamma S_t) \right).$$

This implies that the parameter estimation may be less robust for the model choice (2.4a), due to the failure of linear approximations to the behavior of the sum of squares. There is another source of difficulty in (2.5a): the logarithmic term may be singular at certain trial parameter values. Algorithms which search for a minimum sum of squares in such a case may experience difficulties due to large gradients in the sum of squares near such singular values. A functional form which avoids these difficulties has a clear advantage for parameter estimation purposes. The preceding arguments make it plausible that the Ricker model and its generalizations should be preferred over the Deriso–Schnute family in cases where information is scanty, or noise is large.

3. Comparison of Deriso–Schnute Model, the Generalized Ricker Model, and a Composite Estimation Scheme

The comparison in this section uses the full nonlinear estimation method as outlined in Ludwig et al. (1987). To facilitate the comparison across many different methods and parameter choices, the results are presented in the form of a table, whose construction and significance are explained below. From these comparisons, one can verify that the effect of a choice of a large p in the generalized Ricker scheme is to decrease the variance of the estimator for optimal effort, and often to introduce a bias as well. This observation prompts the choice of a composite estimation method, whereby the accuracy of the estimated optimal effort is first diagnosed. Then if low accuracy is suspected, the choice $p = 2$ is made. This composite scheme appears to be robust.

Procedure

The various estimation schemes were tested for 12 cases. In each case, artificial data were generated using the Deriso–Schnute stock–production model as described in Appendix C, with $\alpha = 0.5$, $\beta = 0.2$, and $\lambda = 0.5$. The quantity λ is defined as follows: the sum of the variances of the pseudo-random sequences $\{w_t\}$ (process errors) and $\{v_t\}$ (observation errors) is denoted by σ^2. Then the parameter λ is defined to be the ratio of the variance of $\{w_t\}$, divided by σ^2. This choice of λ implies equal variances for the sequences $\{w_t\}$ and $\{v_t\}$. The values of σ^2 are given in Table 1 for each of the 12 cases. The other rows in Table 1 specify the effort sequence as given in Fig. 1, the value of q, and the initial stock size. It should be noted that efforts appear only in the combination qE in generating the data. Therefore, q may be thought of as a scale factor which modulates the amplitude of the effort sequence.

For each of these cases, seven estimation schemes were tested. Each test consisted of 100 trials. Each trial consisted of applying the estimation scheme under test to 25 sets of simulated catch and effort data, which were generated using the parameters for that case. The differences between data sets in each trial arose from different pseudo-random sequences of $\{w_t\}$ and $\{v_t\}$.

The Total Least Squares method described in Ludwig et al. (1987) was used for the parameter estimation. Details of the method are summarized in Appendix C. Starting values for the estimated parameters were chosen as follows:

$$(3.1)\quad \hat{\beta}_0 = 1/\max C_t$$

$$(3.2)\quad \hat{q}_0 = 1/\text{median } E_t$$

$$(3.3)\quad \hat{\alpha}_0 = 0.5.$$

Then the estimated optimal effort was computed, and the value of choosing such a constant effort was compared with the value of choosing the optimal constant effort, using the "true" model and parameter values.

TABLE 1. Specification of the 12 cases used to test estimation schemes. Row 2 lists the values of σ^2 used to generate the simulated data, row 3 lists the number of the effort sequence as depicted in Fig. 1, and row 4 lists the corresponding catchabilities. The remaining rows provide the shape parameter for the Deriso–Schnute model and the starting stock size, respectively.

	1	2	3	4	5	6	7	8	9	10	11	12
σ^2	0.05	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.05	0.16
Effort sequence	1	2	2	3	1	3	1	3	1	1	1	1
q	0.94	1	1	1	0.94	0.6	1	0.6	0.63	0.5	0.31	0.5
γ	−1	−0.5	1	1	−1	−1	1	1	1	−1	−0.5	−0.5
S_0	3	1	1	1	3	2	2	1.5	2	3	1	3

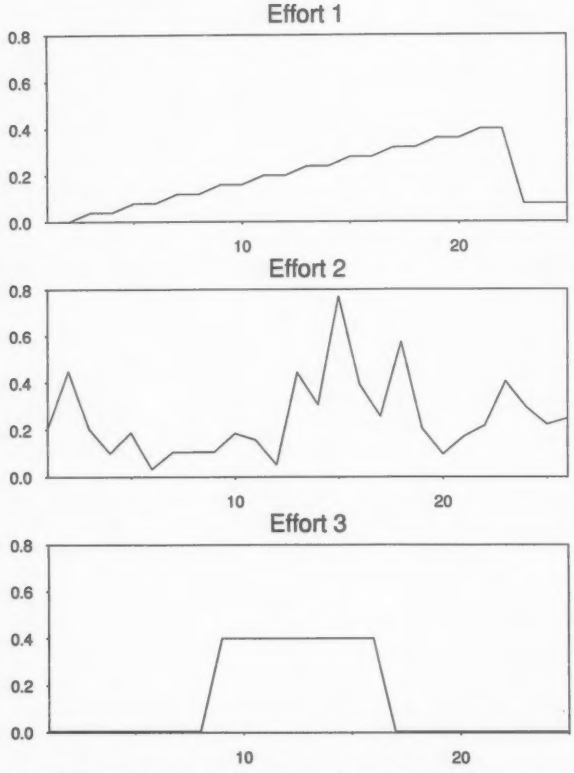

FIG. 1. Three time series of efforts which were employed to generate simulated data for the 12 cases used to test estimation schemes. The middle graph shows a scaled version of the efforts reported for Rock-sole in Hecate Strait, B.C. (Tyler and Macfarlane 1985).

Comparison of the Deriso–Schnute and Generalized Ricker Schemes

Each row of Table 2 corresponds to a case as described above. The entries in that row correspond to seven estimation schemes. The number entered is the median of the observed distribution (over 100 data sets) of the ratio between the value of employing the estimated optimal effort and the value of employing the true optimal effort. The rows in Table 2 are ordered according to the following criterion: first the maximum of all the medians in the row is computed, and then the rows are sorted by decreasing order of the maximum medians. For instance, the entry in the second row has a median of 0.93 corresponding to $p = 3$. This is the maximum of all the medians in that row of the table, but it is less than the maximum of the preceding row. Therefore the upper rows of the table correspond to data sets for which the optimal effort is relatively well estimated, but in the lower rows the estimation schemes do not perform very well. Generally speaking, the differences along rows are not very large in the upper rows, but they become larger in the lower rows.

TABLE 2. Median of the value of using the estimated optimal effort. The results in the column headed "D–S" give the median value obtained by using the "correct" model for the estimation. The results in columns headed "p = ..." give the median value using the generalized Ricker model with the corresponding p parameter. The results in the column labelled "$\lambda = 0$" give the median value corresponding to the choice $\lambda = 0$ and $p = 2$ in the generalized Ricker model. The final two columns give results for combined strategies, as described in the text. The first combined strategy was obtained by estimating the accuracy of q, and the second by estimating the accuracy of the optimal effort.

Case No.	Generalized Ricker model					Combined strategies	
	D–S	$p = 1$	$p = 2$	$p = 3$	$\lambda = 0$	$\Delta^2 q = 2$	$\Delta^2 e = 2$
1	0.93	0.95	0.92	0.88	0.96	0.95	0.95
2	0.89	0.90	0.91	0.93	0.48	0.82	0.89
3	0.83	0.85	0.91	0.92	0.40	0.76	0.86
4	0.89	0.86	0.86	0.67	0.93	0.93	0.91
5	0.79	0.82	0.83	0.84	0.75	0.76	0.81
6	0.80	0.83	0.43	0.29	0.63	0.68	0.75
7	0.80	0.75	0.80	0.83	0.57	0.64	0.82
8	0.74	0.81	0.53	0.16	0.58	0.65	0.72
9	0.53	0.58	0.66	0.64	0.81	0.74	0.72
10	0.48	0.35	0.57	0.63	0.90	0.89	0.82
11	0.33	0.37	0.61	0.47	0.76	0.77	0.78
12	0.46	0.47	0.52	0.46	0.85	0.83	0.75

For the moment, we concentrate on the columns of Table 2 labeled "D–S" (for the Deriso–Schnute model used to generate the data) and "p = 1" to "p = 3" (corresponding to the parameter choice in the generalized Ricker model). The "correct" Deriso–Schnute model performs best only in row 4, and the differences between the first three medians in that row are not very great. In the lower third of the table, where differences along rows are largest and the overall performance of the estimation schemes is poorest, the "correct model" falls far behind the best of the others. Overall, the choice $p = 2$ seems to perform the best. But there are serious exceptions in lines 6 and 8. The choice $p = 2$ shows the most improvement over the others in line 11. These three cases are discussed in more detail below.

Figure 2 shows the distribution of the estimated optimal effort, for the exceptional cases (rows 11, 6, and 8). The pattern is remarkably similar among them. In fact, the results for the best performing estimations also look the same, except that the bias and variance in the estimates are smaller. The bias in effort

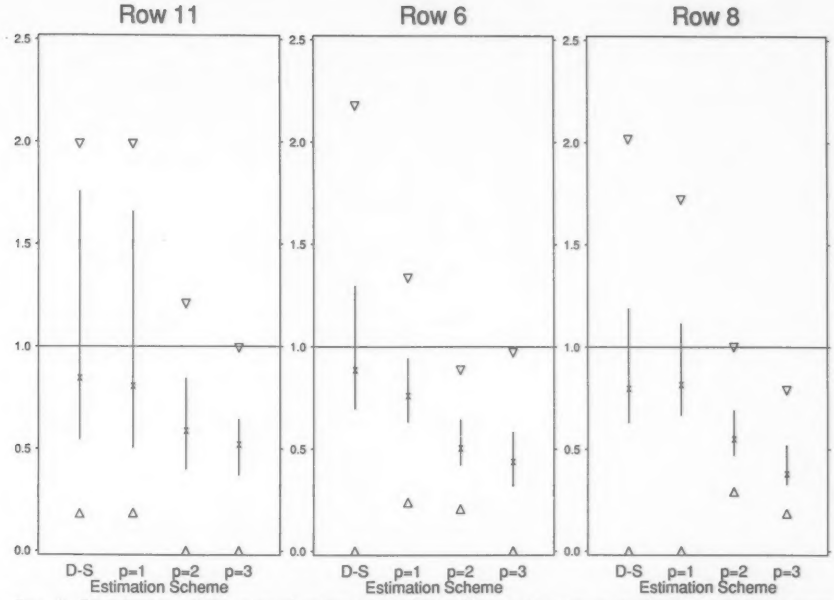

FIG. 2. Distribution of the ratio of estimated optimal effort to the true optimal effort for three cases using the (exact) Deriso–Schnute model and the generalized Ricker model for estimation. The results for the cases are labelled "Row 11," "Row 6," and "Row 8," corresponding to rows in Table 2. Within each trial, the distribution of the estimated optimal effort is shown as a modified boxplot: the "×" marks the position of the median, and the endpoints of the segments mark the quartiles. The triangles indicate the range of the distribution. These boxplots correspond to the Deriso–Schnute model, and three choices of p for the generalized Ricker model. The performance of the Deriso–Schnute model and the generalized Ricker model with $p = 1$ is especially poor in the case of row 11.

is least for the "correct" Deriso–Schnute model, but the spread in the estimates decreases as p increases. In the case of row 11, where $p = 2$ is relatively successful, the $p = 1$ estimate and the Deriso–Schnute estimate are much worse behaved than the cases corresponding to rows 6 and 8. What is different about the data in these three cases? Figure 3 shows the simulated biomass history from which the data were generated. The process errors were set equal to 0 in calculating the data for these plots, but process errors were present in the generated data. The main difference between row 11 and the other cases is that the catchability in row 11 is comparatively small. Consequently the stock was not strongly affected by fishing, and none of the methods performed very well.

Reduction in Bias for $p = 2$

Examination of Fig. 2 and other figures corresponding to other rows in Table 2 reveals that the main problem with the choice $p = 2$ for estimation is the resulting bias in estimated optimal effort. Can this be overcome? We have no final or best answer to this question. However, the results in Ludwig et al. (1987) show that there is an upward bias in estimated optimal effort if the errors in observed efforts are ignored in the estimation. Thus a plausible scheme is to set $p = 2$ in the estimation, but also ignore observation errors by setting $\lambda = 0$. Some results of this scheme are shown in Table 2, under the heading "$\lambda = 0$." There is a striking improvement in the lower rows of the table, but an equally striking loss in some of the upper rows.

Diagnosis of Estimation Difficulties

If we could know in advance whether we are in the top or the bottom portion of the table, we could improve upon all of the preceding schemes by a mixed scheme combining the choices $p = 1$ and $p = 2$, the latter neglecting observation errors. A clue to such a diagnosis is provided in Fig. 3. The data corresponding to row 11 provide little variation in stock size. Therefore we expect the parameter q and the estimated optimal effort to be poorly determined in such a case.

The column in Table 2 labeled "$\Delta^2 q = 2$" shows the effect of a combined strategy, which uses a second difference in estimated q as a diagnostic of lack of information in the data. The first step in the diagnosis is estimation of parameters, fixing $p = 1$ and $\lambda = 0.5$ as before. Then two more parameter estimates are computed, this time constraining the estimated q to differ from the previously estimated q by factors of 3/2 and 2/3, respectively. The details of such constrained estimation are given in Ludwig et al. (1987). If the second difference in estimated q was less than 2, or if the estimation scheme was unable to satisfy the constraint (presumably due to uninformative data), then a problem in estimation was diagnosed, and parameters were estimated by fixing $p = 2$ and $\lambda = 0$. The choice of cutoff 2 in the second difference was determined by examining a range of possible cutoff values. Results were not very sensitive to this choice.

Although some results in the upper rows of Table 2 are degraded by the mixed strategy, there is a considerable gain in robustness, in the sense that none of the results are very bad, although some could be improved somewhat by an optimal choice between the $p = 1$ and $p = 2$ cases.

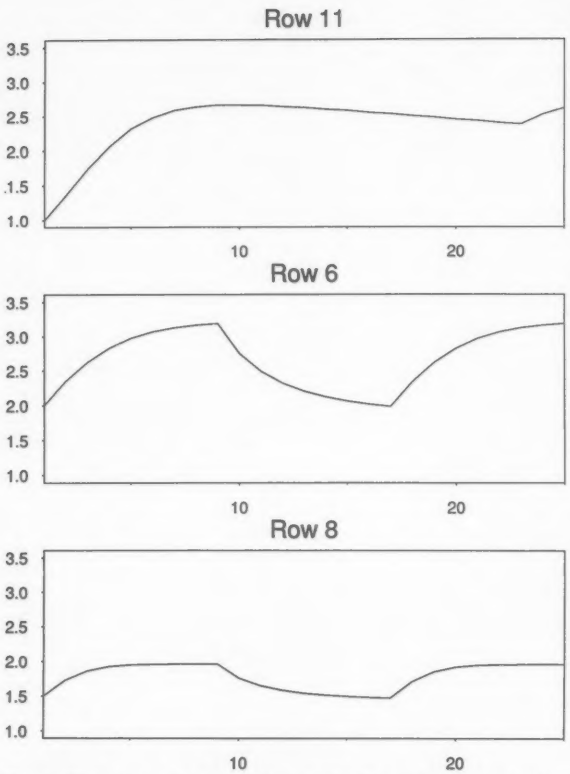

FIG. 3. Time series of nominal biomass for the cases in rows 11, 6, and 8 of Table 1. These plots were generated using the same parameter values and effort sequence as were used to generate the catch data in the respective cases, but the process errors w_t and the observation errors v_t were set equal to zero. It is clear that the efforts used in row 11 had much less effect on the population than those in rows 6 and 8, but row 8 shows a weaker effect than row 6.

The column of Table 2 labeled "$\Delta^2 e = 2$" corresponds to a mixed strategy, where the estimated optimal effort is constrained instead of q. The results are considerably improved in rows 7 and 8, and not much worse in the remaining columns. Therefore it would appear that the mixed strategy with diagnosis depending upon $\Delta^2 e$ is the most robust in providing satisfactory performance over the widest range of input data.

4. Summary and Conclusions

On the basis of the preceding results, and the results in Ludwig and Walters (1985) and Ludwig (1988), a coherent picture emerges. The trade-off between bias and efficiency in estimators is described completely for a simple case in Ludwig (1988). For catch and effort data with large errors and/or low variation in stock size and effort, one is compelled to give up fidelity of the estimation model to the underlying dynamics in order to improve estimation performance. This principle applies to data for the case of age-structured populations as in Ludwig and Walters (1985) or as in Schnute (1985). Moreover, there are important differences even between simple models. The Power model was shown to be inferior to the Ricker model in Ludwig et al. (1987). The present work shows that the family of Deriso–Schnute stock–production models is also generally inferior to the Ricker model for estimation purposes. Within the family of generalized Ricker models, no single model can dominate the others. As was explained at the end of Section 2, one can always gain a bit in the total of bias and variance errors by choosing a value of p which is somewhat larger than the "correct" value.

The most serious problem remaining is to control or somehow correct for the bias introduced by choosing the more efficient estimators. In the present work, a rather ad hoc procedure was adopted, namely to neglect observation errors in effort in the case where such errors were of the same magnitude as the process errors. We would hesitate to recommend such a procedure in general without a more thorough investigation. One should test a variety of plausible estimation schemes on simulated data which are similar to the data to be analysed before accepting any particular scheme. It would also be worthwhile to develop more sophisticated composite estimation schemes involving an array of diagnostic tools.

Our present results do not provide adequate information concerning the utility of data specifying catch by length or weight of individuals instead of the composite data which we have been considering. On the basis of the principles enunciated above, one would not ordinarily expect that the additional information carried in the more detailed data could compensate for the additional complexity required in the estimation scheme. However, there might be exceptions in the case of data which are completely uninformative without the additional information.

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Appendix A. Dependence of the Variance of $\hat{\alpha}$ upon p

We show here that the variance of $\hat{\alpha}$, given by (2.8), is a decreasing function of p. We shall denote the mean of a quantity by placing a bar over it. Thus

$$\bar{X} = \frac{1}{n} \sum_{i=1}^{n} X_i$$

and $\overline{X^2}$ (not to be confused with $\bar{X}^2$) is given by

$$\overline{X^2} = \frac{1}{n} \sum_{i=1}^{n} X_i^2.$$

Lemma

If $0 < X_i < 1$ and if $u_i = \log(\frac{1}{X_i})$, then

$$\text{(A.1)} \quad \sum_{i=1}^{n} u_i \frac{X_i}{X} - \sum_{i=1}^{n} u_i \frac{X_i^2}{\overline{X^2}} > 0.$$

Proof

Let

$$A = \frac{1}{n} \sum_{i=1}^{n} \frac{X_i^2}{\bar{X}}.$$

$A < 1$ since $X_i < 1$ for all i. Let $B = \log(\frac{1}{A})$. Then (A.1) may be written as

$$\text{(A.2)} \quad \sum_{i=1}^{n} (X_i/\bar{X} - X_i^2/\overline{X^2})u_i = \sum_{i=1}^{n} (X_i/\bar{X} - X_i^2/\overline{X^2})(u_i - B)$$

since the mean of the term in parentheses is zero. In the right-hand side of (A.2), if the first factor is positive, so is the second factor, and if the first factor is negative, so is the second factor. Therefore the difference is always positive, which implies (A.1).

Now we proceed to our main objective. We shall demonstrate that Q, given by

$$\text{(A.3)} \quad Q = \frac{\frac{1}{n}\Sigma_{i=1}^{n} (X_i - \bar{X})^2}{\overline{X^2}},$$

is increasing with p, if $X_i = S_i^p$. Since Q is invariant under a scale factor in X or S, we may assume without loss of generality that $S_i < 1$, $i = 1,\ldots,n$. The derivative of Q with respect to p is given by

$$\text{(A.4)} \quad \frac{dQ}{dp} = \frac{2}{p} \frac{\overline{X^2}}{\overline{X^2}} \left[\frac{\overline{uX}}{\bar{X}} - \frac{\overline{uX^2}}{\overline{X^2}} \right].$$

The right-hand side of (A.4) is positive, by the preceding lemma.

Appendix B. Estimation of the Optimal Stock Size

This Appendix details the calculation of the optimal stock size, and the associated exploitation rate. The objective of our estimation scheme is to choose a constant effort, which will maximize the expected present value of future harvest. If we ignore fluctuations in recruitment, this can be done fairly easily on the basis of the assumptions in Section 2.

In view of (2.3), the biomass in any given year is a random function of the stock in the preceding year. This feature complicates the determination and analysis of the optimal strategy. For our present purpose of comparing estimation schemes, this is an unnecessary complication: it is sufficient to assume that the bioamss before harvest is given by

$$\text{(B.1)} \quad B = \tilde{f}(S)$$

where

$$\text{(B.2)} \quad \tilde{f}(S) = f(S)e^{\frac{1}{2}\sigma_w^2}$$

and where S is the number of spawners in the preceding year. With this assumption, the model is reduced to a deterministic one, and the optimal strategy consists in choosing an optimal spawning stock size, and holding the spawners at that level in all future years.

If the initial biomass is B_0 and we employ a strategy of holding the biomass after harvest at a constant size S, then the first catch is

$$\text{(B.3)} \quad C_0 = B_0 - S.$$

In subsequent years, the expected biomass before harvest is given by (B.1). In order to maintain the stock at equilibrium, the catch C must equal $B - S$; thus

$$\text{(B.4)} \quad C = \tilde{f}(S) - S.$$

Future catches are multiplied by a factor of δ for each year elapsed. Thus we determine S to maximize

$$\text{(B.5)} \quad V = B_0 + \frac{1}{1-\delta} [\delta\tilde{f}(S) - S].$$

If we set

$$\text{(B.6)} \quad g(S) = \delta\tilde{f}(S) - S,$$

then V is maximized if we maximize g with respect to S. For the purpose of estimating the optimal effort, estimated parameter values are employed in computing $f(S)$. Once S has been determined, the corresponding value of E is obtained from (B.2) and (2.2).

In all of the stock production models of Section 2, the function f is of the form

$$\text{(B.7)} \quad \tilde{f}(S) = Sk(\hat{\beta}S)$$

where the function k depends upon the parameters $\hat{\alpha}$, γ, p, etc., but not $\hat{\beta}$. Therefore if we introduce $\tau = \hat{\beta}S$, then we may equally well maximize

$$\text{(B.8)} \quad \hat{\beta}g(S) = \delta\tau k(\tau) - \tau.$$

The optimal $\hat{\tau}$ satisfies

$$\text{(B.9)} \quad \delta(k(\hat{\tau}) + \hat{\tau}k'(\hat{\tau})) = 1.$$

It is clear that $\hat{\tau}$ is independent of $\hat{\beta}$.

Exploitation Rate

The exploitation rate R which corresponds to the optimal S is defined as

$$\text{(B.10)} \quad R = C/B$$

where C and B are the catch and biomass which correspond to $\hat{\tau}$. The expected biomass at such a stock size is

(B.11) $B = Sk(\hat{\tau})$.

The stock will be at equilibrium at S only if $B - C = S$. Therefore

(B.12) $$\frac{1}{1 - R} = k(\hat{\tau}).$$

It is clear from the preceding that the optimal exploitation rate depends only upon the estimate $\hat{\alpha}$. Therefore to a first approximation the variance of R is proportional to the variance of $\hat{\alpha}$.

Appendix C. Nonlinear Estimation Method

This Appendix describes the estimation procedure employed for the comparisons of Section 3. We are concerned with time series of observations of fishing effort E_t and catch C_t ($t = 1,\ldots,n$) as in Section 2. However, the effort is assumed to be observed with error: the "effective effort" e_t which appears in the catch equation below is assumed to be related to the observed effort by

(C.1) $e_t = E_t e^{v_t}$

where $\{v_t\}$ is a sequence of independent normally distributed random variables with mean 0 and variance σ_v^2. We assume that the stock biomass before harvest B_t is related to the catch by

(C.2) $C_t = B_t(1 - e^{-qe_t})$

in analogy with (2.1). Equation (2.3) is unchanged:

(C.3) $B_{t+1} = f(S_t)e^{w_t}$.

Specific forms for the recruitment function $f(S)$ are given by (2.4a) and (2.4b). We shall refer to the $\{v_t\}$ as "observation errors" and the $\{w_t\}$ as "process errors." The ratio of their variances determines a parameter λ, defined by

(C.4) $$\lambda = \frac{\sigma_v^2}{\sigma_v^2 + \sigma_w^2}.$$

In the present work, we have always chosen $\lambda = 0.5$, both to generate trial data and for estimation purposes.

The "total least squares" method consists in augmenting the process error sum of squares with a sum of squares of estimated observation errors in the effort. The total sum of squares T is given by

(C.5) $$T = \frac{1}{1-\lambda} \sum_{t=1}^{n-1} w_t^2 + \frac{1}{\lambda} \sum_{t=1}^{n} v_t^2.$$

In this approach, the v_t are regarded as parameters to be estimated, rather than random variables. Thus the parameters q, α, β, $v_1,\ldots,v_n$ are chosen to minimize T.

Interaction of Temperature and Photoperiod in the Regulation of Spawning by American lobsters (*Homarus americanus*)

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Aiken, D. E., and S. L. Waddy. 1989. Interaction of temperature and photoperiod in the regulation of spawning by American lobsters (*Homarus americanus*). Can. J. Fish. Aquat. Sci. 46: 145–148.

Various studies have yielded conflicting information on the role of photoperiod in the regulation of spawning by the American lobster (*Homarus americanus*). Some have indicated a requirement for short days followed by long days, while others have suggested that there is no photoperiod requirement. To resolve the question of photoperiod control, we exposed mature female lobsters to various combinations of temperature and photoperiod for 1 yr starting in December and found that there was no requirement for spring photoperiod under local winter seawater temperature (0–5°C), but when winter seawater temperature was maintained at 10–17°C, spawning came under the influence of spring photoperiod. It therefore appears that vitellogenesis and spawning of nearshore lobster populations are normally regulated by seasonal seawater temperature but that photoperiod can assume a regulatory role if the winter seawater temperature remains abnormally high.

Diverses études ont donné des résultats contradictoires sur le rôle de la photopériode dans la régulation de la ponte du homard américain (*Homarus americanus*). Certaines indiquent la nécessité de jours courts suivis par des jours longs, tandis que d'autres indiquent que la photopériode n'a aucune influence. Pour déterminer le rôle de la photopériode, nous avons exposé des homards femelles à diverses combinaisons de température et de photopériode. L'expérience, commencée en décembre, a duré 1 an, et n'a révélé aucune exigence liée à la photopériode printanière dans des conditions de température correspondant à celles de l'eau de mer en hiver dans la région (0–5°C). Par contre, quand la température de l'eau de mer en hiver était maintenue à 10–17°C, la ponte était influencée par la photopériode printanière. Il semble donc que la vitellogénèse et la ponte des populations littorales de homard soient normalement régulées par la température saisonnière de l'eau de mer, mais que la photopériode puisse jouer un rôle régulateur si la température de l'eau en hiver reste anormalement élevée.

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Nelson et al. (1983), working on the west coast of the United States (California) with lobsters transported from the east coast (Massachusetts), concluded that spawning of wild American lobsters (*Homarus americanus*) requires exposure to short days (8 h light : 16 h dark) (LD 8:16) followed by long days (16 h light : 8 h dark) (LD16:8). However, our studies with lobsters from the east coast of Canada (Gulf of St. Lawrence) held in typical winter seawater temperatures revealed no correlation between long-day onset (LDO) and time of spawning (Aiken and Waddy 1985a, 1986a). These animals spawned in July and were unaffected by photoperiods that ranged from continuous short days to continuous long days and even included short daylengths that *decreased* during the spring period when LDO was presumably required.

In attempting to explain these anomalies, Nelson (1986) offered two suggestions: (1) lobsters from the Massachusetts coast might be genetically distinct from their counterparts in the Gulf of St. Lawrence or (2) the basic photoperiod requirement might be modified by the low winter temperature common to nearshore waters of the western North Atlantic. In this study we evaluate the effect of winter temperature on the gonadal response of mature female lobsters and thereby answer the question of genetic difference.

Methods

Mature female lobsters (cement gland stages 1.0 or greater) (Aiken and Waddy 1982) were selected in September of 1984 and 1985 from the commercial catch of fishermen at Miminegash, P.E.I., in the southern Gulf of St. Lawrence. Chelipeds were immobilized with rubber bands and the lobsters were packed in chilled insulated boxes and transported to the Biological Station at St. Andrews, N.B., where they were held communally in a 4-m^2 tank with individual shelters. Flow-through seawater at local temperature (Aiken and Waddy 1985a) was supplied at 20–30 L/min. Natural photoperiod was available through an adjacent exterior window. During the 3-mo holding period the lobsters were fed a rotating daily diet of squid, cod, shrimp, mackerel, mussels, and herring.

Photoperiod–temperature experiments were conducted in three light-tight rooms in which lobsters were held individually in glass-fronted cubicles of 0.21 m^2. Flow-through seawater (mean salinity 31 ppt) was filtered through sand and supplied at 2 L/min. Temperature control was achieved by blending unheated and heated local seawater to prescribed temperature in a mixing valve. Photoperiod in each room was maintained

TABLE 1. Experimental design and distribution of American lobsters.

	LDO 17 April	LDO 12 June	No LDO
High winter temperature (9.8 – 16.8°C)	Group 1A (N = 16)	Group 1B (N = 15) Group 2A (N = 24)	Group 1C (N = 20)
Low winter temperature (0 – 14°C)		Group 2B (N = 24)	Group 2C (N = 21)

with electronic time clocks controlling an incandescent lamp that provided approximately 22 1m/m² to each cubicle. Feeding was as indicated above.

Experiment 1. Photoperiod and Elevated Winter Temperature

To determine whether the responses of Gulf of St. Lawrence lobsters were similar to those of Massachusetts lobsters, we assigned mature Gulf females to the temperature and photoperiod conditions that Nelson et al. (1983) used with lobsters from Massachusetts.

On 21 December 1984, 51 of these females were randomly distributed among three groups (Table 1) maintained between 9.8 and 16.8°C according to the schedule in Nelson et al. (1983, p. 941). These groups were initially held in short-day photoperiod (LD 8:16). The change to LD 16:8 (LDO) occurred on 17 April in Group 1A and 12 June in Group 1B. In Group C there was no LDO; short days continued until the end of the study.

Lobsters were examined weekly for spawning or oocyte resorption (dark green hemolymph visible through the sternal membrane of the abdomen). The experiment ran for 1 yr and was terminated on 20 December 1985.

Experiment 2. Photoperiod and Low Winter Temperature

To determine whether low winter temperature could modify the effects of photoperiod, 69 mature female lobsters were divided into three groups (Table 1). Group 2A was held at elevated temperature (replicate of Group 1B), while Groups 2B and 2C were held at low winter temperature typical of nearshore lobster habitat (varying seasonally from 0 to 14°C (Aiken and Waddy 1985a)). Group 2B was exposed to LDO on 12 June whereas Group 2C was maintained on short days throughout the experiment (photoperiod complement of Group 1C).

Lobsters were examined weekly for spawning or oocyte resorption. The experiment began on 21 December 1985 and was terminated in October 1986 after all females had either spawned or resorbed their oocytes. Massive oocyte resorption was recognized by the dark green hemolymph visible through the sternal membranes and the degenerated condition of the cement glands.

Results

Experiment 1. Photoperiod and Elevated Winter Temperature

LDO 17 April (Group 1A, N = 16)

Nine females (56%) spawned. Mean date of spawning was 21 July (calendar day 202, 94 d post-LDO). Four females (25%) resorbed their oocyte vitellin. Three females neither spawned nor resorbed.

LDO 12 June (Group 1B, N = 15; Group 2A, N = 24)

In Group 1B, six females (40%) spawned. Mean date of spawning was 25 September (calendar day 268, 105 d post-LDO). Three females (20%) resorbed their oocyte vitellin and one female (7%) molted on 21 October. Five females neither spawned, resorbed, nor molted. In Group 2A, 16 females (67%) spawned and four (17%) resorbed their oocyte vitellin. Mean date of spawning was 17 September (calendar day 260, 97 d post-LDO). Four females did not spawn or resorb. There was no significant difference in spawning times of Groups 1B and 2A.

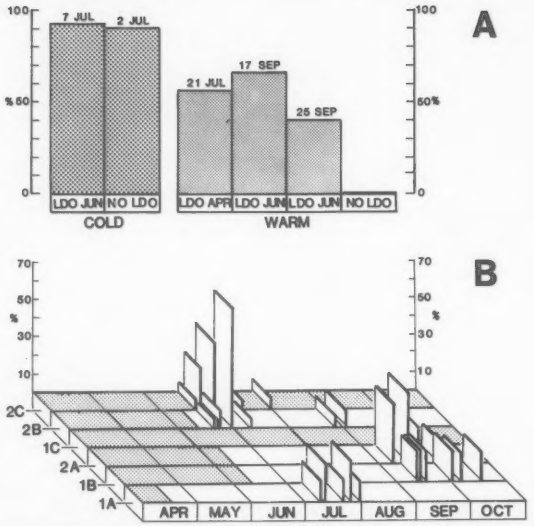

FIG. 1. Effect of low winter seawater temperature on spawning of American lobster in various photoperiod treatments. (A) Spawning incidence (percent of total females) and mean spawning date following a winter period in cold (<5°C) or warm (>10°C) seawater. Mean spawning date is earlier, spawning incidence is higher, and LDO is irrelevant after a winter period of low temperature. (B) Spawning incidence (percent of total that spawned in each group) relative to LDO (indicated by transition from stippled to open horizontal bar) following a cold or warm winter period. Following a cold winter, females spawn irrespective of time of LDO (Groups 2B and 2C). Following a warm winter, females spawn at a set time after LDO and fail to spawn in the absence of LDO (compare Groups 1C and 2C).

No LDO (Group 1C, N = 20)

No females spawned. Eleven (55%) resorbed their oocyte vitellin. Three (15%) molted (5 October, 18 October, and 19 December). Six females neither resorbed nor molted.

Mean day of spawning in Groups A and B (LDO 17 April and 12 June, respectively) was significantly different ($P < 0.001$).

Experiment 2. Photoperiod and Low Winter Temperature

LDO 12 June (Group 2B, N = 24)

Twenty-two females (92%) spawned in Group 2B. Two (8%) resorbed their oocyte vitellin. Mean date of spawning was 7 July (calendar day 188, 25 d post-LDO).

No LDO (Group 2C, N = 21)

Nineteen females (90%) spawned in Group 2C. One (5%) resorbed its oocyte vitellin. One female neither spawned nor resorbed. Mean data of spawning was 2 July (calendar day 183).

Mean spawning dates of Groups 2B and 2C were statistically similar in spite of their different photoperiod exposure. Both

TABLE 2. Spawning responses of American lobsters to LDO following either a warm or a cold winter period.

Group spawning	High winter temperature (>9.8°C)				Low winter temperature (<5°C)			
	N	%	Date	SD	*N*	%	Date	SD
LDO April	16	56	21 July 94[a]	14				
LDO June	39	53	21 Sept. 100[a]	13	24	92	7 July 25[a]	32
No LDO	21	0			20	90	2 July	15

[a]Days past LDO.

differed significantly from 2A and 1B, in which the 12 June LDO occurred at elevated temperature ($P < 0.001$).

Discussion

Low winter temperature clearly dominates spring photoperiod in the control of spawning. At winter temperatures typical of nearshore habitat throughout the range of the American lobster, lobsters are able to spawn at the normal time (July) without any exposure to LDO (Group 2C, Fig. 1B; Table 2). However, if winter water temperatures remain abnormally high, lobsters will not spawn without the stimulus of long-day photoperiod (Fig. 1B, Group 1C). In other words, elevated winter temperature imposed a strong requirement for LDO, but low winter temperature eliminated this requirement. These results are supported by our earlier low temperature experiments in which the ovarian cycle proved unresponsive to photoperiod regimes imposed as much as 10 mo before spawning and was unaffected by photoperiods that were short, long, steadily decreasing, or delayed increasing (Aiken and Waddy 1985a, 1986a).

However, when winter seawater temperature is maintained above approximately 10°C, photoperiod becomes influential. Short (or possibly unchanging) spring daylengths prevent spawning, and the requirement for LDO (or possibly a simple increase in daylength) becomes absolute (Nelson et al. 1983). Thus these experiments clarify the photoperiod–temperature interaction and explain the conflicting results reported from similar studies on the east and west coasts of North America (Aiken and Waddy 1985a, 1985b, 1986a; Nelson 1986; Nelson and Hedgecock 1985).

These experiments also seem to resolve the question of genetic differences. In an earlier study (unpubl. data), we replicated the temperature–photoperiod conditions of Nelson et al. (1983) using lobsters from the coastal waters of Massachusetts and lobsters from the southern Gulf of St. Lawrence. Gaffkemia decimated the Massachusetts stock and compromised the study, but we obtained sufficient information to discern that both stocks responded in the same way to the experimental conditions. In Experiment 1 of this study, we repeated that earlier work but without the Massachusetts lobsters. Again the Gulf lobsters responded exactly as did the Massachusetts lobsters used by Nelson et al. (1983).

Thus the accumulating evidence indicates that the different responses to photoperiod that have been reported for these two stocks are a result of their winter seawater temperature history, not their genetic differences. Final ovarian maturation and spawning in both stocks appear to require an extended period of temperature below 5°C followed by an increase to approximately 10°C (Aiken and Waddy 1986b, and unpubl. data). Alternatively, if the seawater temperature has remained above approximately 10°C throughout the winter, the requirement shifts to an extended period of short days (80 d according to Nelson (1986) and Nelson et al. (1988a)) followed by an increase to long (or longer?) days. However, it is important to note that these conditions are associated with a higher incidence of reproductive failure and a lower incidence of spawning (Fig. 1A).

The reduced incidence of spawning and increased incidence of ovary resorption under elevated temperature and photoperiod control (Fig. 1A) suggest that these conditions present a less than optimum environment for ovary maturation and spawning, certainly less favorable than is provided by low winter temperature. Similar problems were noted in elevated winter temperature studies reported by Hedgecock (1983), Nelson (1986), and Nelson et al. (1983), and there was also a high incidence of egg loss following spawning, further evidence for a suboptimal reproductive environment. Warm water and long daylengths favor the molting system, a fact commonly invoked to explain reduced reproductive performance, but Nelson (1986) and Nelson et al. (1988a, 1988b) dismissed this, preferring instead an hypothesis that the physiological milieu is receptive to LDO stimulus only in proximity to the molt.

Temperature above 8°C would be available to lobsters throughout the winter in some areas of the continental slope, a fact that led Nelson (1986) to suggest that photoperiodic control of spawning evolved in offshore lobsters during the Pleistocene and was carried shoreward as the animals subsequently colonized the submerging continental coastline. One of the more interesting aspects of this study is the facultative nature of the control mechanism: at low temperatures the system is regulated by temperature, but at elevated temperature it is regulated by photoperiod. Temperature effects on photoperiodic systems are known in insects (Beck 1980), but temperature extremes generally uncouple the photoperiodic control mechanism. However, in some saturniid moths the pupal diapause can be terminated by either temperature or photoperiod (Mansingh and Smallman 1966, 1967, 1971).

Although the temperature–photoperiod problem appears to be resolved, it is far from clear how this facultative regulatory system translates into control of final vitellogenesis, ovulation, and spawning and how these events are related to and affected by the molt cycle.

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Biochemical Genetic Comparison of Sockeye Salmon and Kokanee, the Anadromous and Nonanadromous forms of *Oncorhynchus nerka*

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Twenty-three anadromous (sockeye salmon) and nonanadromous (kokanee) *Oncorhynchus nerka* populations were sampled from throughout British Columbia and examined electrophoretically at three to five polymorphic loci to test whether the forms represent distinct genetic lineages or whether they are polyphyletic. Sockeye and kokanee which spawn sympatrically in three different lake systems were also examined to determine whether the two forms belong to a single panmictic population. Our results support the hypothesis that sockeye and kokanee are polyphyletic. No genetic characters were found by which the forms could be separated consistently. Greater differences exist among *O. nerka* populations from different drainages than between sockeye and kokanee forms. Sympatric sockeye and kokanee were significantly different in all systems examined, demonstrating that genetic differences can persist in the absence of geographic barriers to gene flow. While sympatric sockeye and kokanee were genetically divergent, they showed greater genetic similarity to one another (in allele frequency and/or allele types) than they did to their own forms in neighbouring lakes. We argue that this genetic similarity between sympatric forms is the result of sympatric divergence of sockeye and kokanee.

Vingt-trois populations de saumons rouges anadromes et de saumons kokanis non anadromes (*Oncorhynchus nerka*) réparties dans toute la Colombie-Britannique ont été échantillonnées dans le but de faire un examen électrophorétique portant sur de trois à cinq locus polymorphes afin de déterminer si ces formes représentaient des lignées génétiques distinctes ou si elles étaient polyphylétiques. Des saumons rouges et kokanis frayant de façon sympatrique dans trois bassins versants de lacs ont aussi été examinés pour déterminer s'il s'agissait de deux formes appartenant à une même population panmictique. Les résultats obtenus appuient l'hypothèse voulant que les saumons rouges et kokanis soient polyphylétiques. Aucun des caractères génétiques trouvés ne permettaient d'isoler les formes de façon constante. Il existait de plus importants écarts entre des populations de *O. nerka* de bassins versants différents qu'entre les formes rouges et kokanis. Les saumons rouges et kokanis sympatriques différaient de façon significative dans tous les bassins étudiés, ce qui montre que des écarts génétiques peuvent persister en l'absence de barrières géographiques au transfert de gènes. Les saumons rouges et kokanis sympatriques étaient génétiquement divergents, mais présentaient une plus grande similitude génétique l'un envers l'autre (fréquence des allèles ou type d'allèles) qu'avec leur propre forme se trouvant dans des lacs avoisinants. Les auteurs soutiennent que cette similitude génétique entre les formes sympatriques résultent de la divergence sympatique des saumons rouges et kokanis.

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The Pacific salmon *Oncorhynchus nerka* occurs in two distinct forms, the anadromous sockeye salmon and the nonanadromous kokanee. Sockeye salmon typically spend their first year of life (sometimes longer) in a lake before migrating to the ocean whereas kokanee remain in a lake throughout their lifetime. Sockeye usually attain at least twice the length of kokanee at maturity, largely because of the difference in productivity between the marine and freshwater environments (Foerster 1968). Sockeye salmon and kokanee populations occur either together or separately (Ricker 1940; Nelson 1968a). Where they occur sympatrically, spawning usually occurs in separate localities and at different times (Ricker 1940; Nelson 1968a). In a few localities, sockeye and kokanee spawn in the same place at the same time (Ricker 1940; Hanson and Smith 1967; McCart 1970).

It is generally accepted that kokanee have originated from sockeye on numerous independent occasions (Ricker 1940, 1959, 1972; Nelson 1968a; Behnke 1972). This conclusion is supported through an examination of the distribution of the two forms (Ricker 1940; Nelson 1968a) and by observations that nonanadromous populations of *O. nerka* have appeared after sockeye salmon were introduced to lakes previously barren of the species (Ricker 1959, 1972; Scott 1984). It seems more probable that kokanee in Japan, Siberia, and western North America (including Vancouver Island) have arisen largely from the marine dispersal of sockeye salmon following the retreat of the Wisconsin continental ice masses rather than solely through the freshwater dispersal of kokanee.

There is evidence of genetic divergence between sympatric sockeye and kokanee populations (Nelson 1968b; McCart 1970)

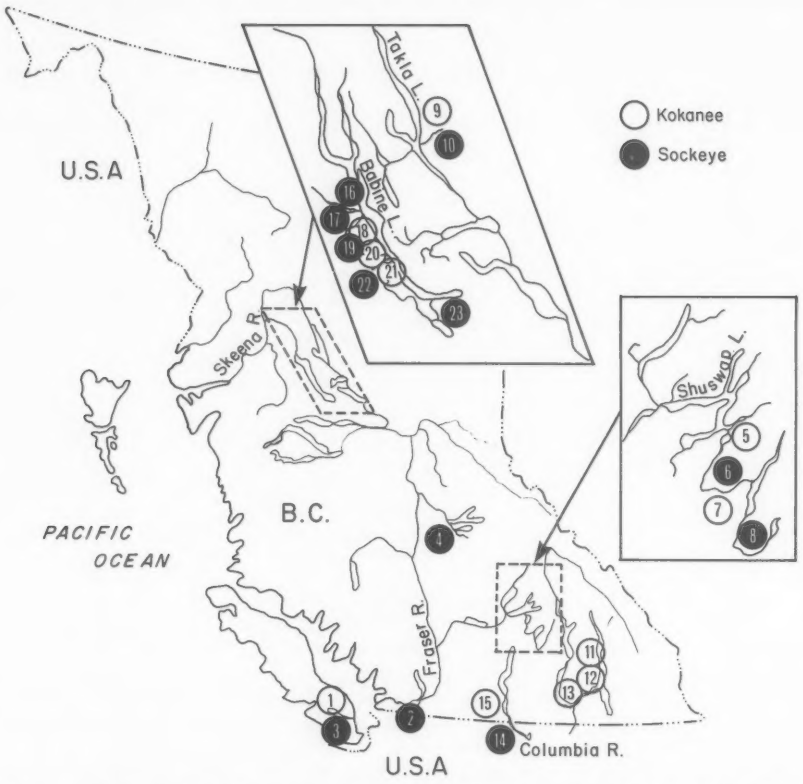

FIG. 1. Location and form of *O. nerka* populations sampled for electrophoresis. Population numbers correspond to those in Table 1.

and various mechanisms have been proposed to account for this. Selective pressures in freshwater and marine environments probably differ greatly, promoting genetic differentiation between the forms (Ricker 1940). Genetic differentiation is further promoted by the tendency of sympatric sockeye and kokanee to spawn in separate locations at different times, limiting gene flow between the forms (Ricker 1940). Where spawning is coincident, gene flow is probably greatly restricted through assortative mating by form, associated with the great size difference between mature sockeye and kokanee (Hanson and Smith 1967; McCart 1970; Foote and Larkin 1988). Sockeye males mate almost exclusively with sockeye females whereas kokanee males mate either with kokanee females or attempt to fertilize the eggs of sockeye females by "sneaking."

In this study, the results of an electrophoretic comparison of sympatric and allopatric sockeye and kokanee populations in British Columbia are used to address questions concerning the recent ancestry and genetic similarity of the two forms. If differentiation has been recent, sockeye and kokanee should be very similar genetically. If one or both forms have given rise to the other form on numerous occasions, then sockeye and kokanee should be genetically interrelated rather than members of two distinct genetic lineages. Additionally, if kokanee have arisen in sympatry with sockeye, sympatric sockeye and kokanee may be expected to be genetically more similar to each other than they are to their respective forms in other lakes.

We sampled sockeye and kokanee from throughout British Columbia, paying particular attention to three localities where the forms spawn sympatrically. Sympatric genetic differentiation would seem to be the least likely in these locations because of the great opportunity for gene flow between the forms. Genetic differentiation in these localities would suggest that mechanisms in addition to segregation to different spawning grounds are important in the genetic differentiation of salmonid populations.

Methods

Kokanee and sockeye were each collected from 11 and 12 localities, respectively, in British Columbia (Fig. 1; Table 1). Five populations were sampled annually for 2 or 3 yr. All samples, with the exceptions of those from Cultus and Cowichan lakes and Okanogan River, were collected with gaffs and small seines in streams where fish were spawning. Sockeye smolts were collected by trap in the outlet of Cultus Lake. Kokanee were captured with a midwater trawl in Cowichan Lake (Rutherford et al. 1988). Electrophoretic data for the Okanogan River sockeye were taken from Utter et al. (1984). Where spawning populations were small (<1000 fish), mainly moribund or freshly dead specimens were collected. Where sockeye and kokanee spawned sympatrically, there were distinguished by size; most sockeye were greater than 45 cm fork length whereas kokanee ranged from 16 to 28 cm. Jack sockeye (precocious

TABLE 1. Allele frequencies at polymomrphic loci for sockeye and kokanee populations sampled in British Columbia. *K* = kokance; *S* = sockeye.

			Pgm-1		*Pgm-2*		*Ldh-4*				*Gl-2*		*Alat*				
Site	Form	Year	100	*N*	100	*N*	100	115	85	*N*	100	*N*	91	100	108	95	*N*
Coastal populations																	
1. Cowichan L.	K	85	0.460	150	0.840	150	1.000	0.000	0.000	150	—	—	—	—	—	—	—
2. Cultus L.	S	85	0.147	158	0.837	95	1.000	0.000	0.000	99	—	—	—	—	—	—	—
3. Cheewhat L.	S	85	0.375	100	0.865	100	1.000	0.000	0.000	100	—	—	—	—	—	—	—
Upper Fraser River populations																	
4. Horsefly R.	S	85	0.624	93	0.805	100	1.000	0.000	0.000	100	0.995	96	0.330	0.569	0.000	0.101	94
5. Eagle R.	K	86	–348	99	0.828	99	1.000	0.000	0.000	99	1.000	99	0.505	0.111	0.354	0.030	99
6. L. Shuswap R.	S	83	0.414	99	0.842	101	0.995	0.005	0.000	97	1.000	80	0.172	0.754	0.030	0.045	67
7.	K	83	0.132	76	0.842	76	0.987	0.006	0.006	77	1.000	78	0.355	0.158	0.480	0.007	99
	K	86	0.142	53	0.783	53	0.991	0.009	0.000	58	—	—	0.336	0.052	0.586	0.026	58
8. M. Shuswap R.	S	86	0.583	144	0.870	142	0.993	0.007	0.000	145	1.000	141	0.132	0.688	0.066	0.115	144
9. Takla L.	K	82	0.357	77	0.903	77	1.000	0.000	0.000	76	—	—	—	—	—	—	—
	K	83	0.340	381	0.865	381	1.000	0.000	0.000	300	—	—	—	—	—	—	—
	K	85	0.367	124	0.846	123	1.000	0.000	0.000	124	0.992	119	0.721	0.119	0.000	0.160	122
10. Takla L.	S	83	0.467	83	0.837	83	1.000	0.000	0.000	79	—	—	—	—	—	—	—
	S	85	0.588	97	0.813	96	1.000	0.000	0.000	97	1.000	100	0.149	0.819	0.000	0.032	94
Columbia River populations																	
11. Meadow Cr.	K	83	0.586	151	0.870	151	0.993	0.003	0.003	151	—	—	—	—	—	—	—
12. Redfish Cr.	K	83	0.595	42	0.964	42	0.977	0.023	0.000	38	—	—	—	—	—	—	—
13. Arrow L.	K	85	0.575	100	0.910	100	1.000	0.000	0.000	98	—	100	0.360	0.615	0.000	0.025	100
14. Okanogan R.	S	—	0.493	71	0.782	71	1.000	0.000	0.000	72	0.995	62	0.285	0.590	0.007	0.000	72
15. Skaha L.	K	83	0.284	88	0.875	88	1.000	0.000	0.000	85	1.000	—	—	—	—	—	
Skeena River populations (Babine Lake)																	
16. Fulton R.	S	85	0.158	291	0.776	290	0.973	0.017	0.010	293	—	—	—	—	—	—	—
17. Tachek Cr.	S	85	0.290	50	0.800	50	0.940	0.010	0.050	50	—	—	—	—	—	—	—
18.	K	85	0.238	103	0.607	103	0.861	0.000	0.139	102	1.000	35	0.500	0.390	0.000	0.110	41
19. Pierre Cr.	S	84	0.215	100	0.735	100	0.968	0.016	0.016	94	—	—	—	—	—	—	—
	S	85	0.202	94	0.766	94	0.953	0.010	0.036	92	0.989	91	0.529	0.382	0.006	0.082	85
20. Pierre Cr.	K	84	0.270	111	0.698	111	0.850	0.000	0.150	110	—	—	—	—	—	—	—
	K	85	0.228	114	0.702	1114	0.870	0.004	0.126	115	0.980	74	0.649	0.299	0.000	0.052	77
21. Twain Cr.	K	85	0.239	92	0.678	101	0.865	0.005	0.130	95	—	—	—	—	—	—	—
22.	S	85	0.214	103	0.830	103	0.971	0.010	0.019	103	—	—	—	—	—	—	—
23. Pinkut Cr.	S	85	0.231	193	0.725	193	0.972	0.005	0.025	198	—	—	—	—	—	—	—

TABLE 2. Enzymes and tissues used to investigate genetic variation in sockeye salmon and kokanee. Buffers used were an amine-citrate buffer (AC) described by Clayton and Tretiak (1972), a Tris, citric acid, lithium hydroxide, and boric acid buffer (RW) described by Ridgway et al. (1970), and a Tris, boric acid, EDTA buffer (MF) described by Markert and Faulhaber (1965).

Enzyme	Tissue	Locus	Buffer
Aspartate aminotransferase	Eye	*Aat-1, 2*	AC
Adenosine deaminase	Muscle	*Ada-2*	AC
Alanine aminotransferase	Muscle	*Alat* (*Gpt-2*)	MF
Peptitdase (glycyl leucine substrate)	Eye	*Gl-2*	MF
Lactate dehydrogenase	Liver	*Ldh-4*	RW
Phosphoglucoisomerase	Muscle	*Pgi*	RW
Phosphoglucomutase	Heart	*Pgm-1*	AC
Phosphoglucomutase	Muscle	*Pgm-2*	RW
Sorbitol dehydrogenase	Liver	*Sdh*	RW
Superoxide dismutase	Muscle	*Sod*	RW

males) were rare in the populations sampled and were greater than 30 cm fork length.

Heart, liver, eye, and muscle tissues were collected from freshly killed fish, or rarely, from those recently dead as judged from redness of the gills. Samples were placed on ice immediately and frozen as quickly as possible, usually within 12 h of collection, and stored at −40°C until assayed. Samples were assayed electrophoretically using standard extraction and gel techniques (e.g. May et al. 1979). The system of nomenclature suggested by Allendorf and Utter (1979) was used to designate loci and alleles (Table 2); the same nomenclature has been used previously for sockeye salmon populations (Grant et al. 1980; Utter et al. 1984; Wilmot and Burger 1985; Quinn et al. 1987). A few samples were assayed at 24 loci but the majority were assayed at a subset of four or eight loci known to be polymorphic in at least some sockeye and kokanee populations. All 27 samples were assayed at *Pgm-1*, *Pgm-2*, *Ldh-4*, and *Aat-3* and 16 were also assayed at *Ada-2*, *Pgi*, and *Sdh*. In addition, 12 of these samples were reassayed at *Gl-2* and *Alat* (previously designated *Gpt-2*; this locus had been difficult to score reliably

until a new staining procedure was developed by P. Aebersold (Northwest and Alaska Fisheries Center, 2725 Montlake Boulevard East, Seattle, WA 98112, pers. comm. to C. C. Wood)).

Differences in genotype frequencies between years and among populations were tested by likelihood ratio (i.e. *G*-test, Sokal and Rohlf 1981) for individual loci and all loci considered simultaneously (using modified critical values for multiple comparisons). Genotype frequencies within populations were compared with those expected under Hardy–Weinberg equilibrium using the chi-square goodness-of-fit test. Allele frequencies at either three to five polymorphic loci, depending on the samples, were used to construct similarity phenograms using the unbiased genetic identity statistic (Nei 1978) and the unweighted pair group methods (Sneath and Sokal 1973).

Variation in allele frequencies (arcsine square-root transformed) was investigated using Method 3 of Henderson (1953) for the following analysis of variance (ANOVA) model:

$$Y_{ijklm} = \mu + T_i + D_j + T \times D_{ij} + L_{jk} + S_{jkl} + e_{ijklm}$$

where Y_{ijklm} is the transformed value of the individual observation of allele frequency, μ is the overall mean value, T_i is the fixed effect of life history type (i = 1–2, sockeye or kokanee), D_j is the random effect due to drainage (j = 1–4, Columbia, Skeena, Upper Fraser, and Coastal, including Lower Fraser River and Vancouver Island), $T \times D_{ij}$ is the effect of the interaction between type and drainage, L_{jk} is the random effect of lake within drainage, S_{jkl} is the random effect of spawning site within lake, and the error term e_{ijklm} is the effect of annual variability within spawning sites.

The significance of differences in allele frequencies between sockeye and kokanee was examined by an *F*-test of the "form" mean square divided by the "form–drainage" interaction, each with a single degree of freedom due to the unbalanced design. All other effects were considered random, and their individual contributions to the total phenotypic variance were estimated by division of the appropriate variance component by the sum of the drainage, type–drainage interaction, lake, site, and error variance components.

Allele frequency variation was also examined in a completely nested gene diversity analysis (GDA) (Nei 1973; Chakraborty 1980). In this model, sockeye and kokanee spawning sympatrically were treated as different "forms" of *O. nerka*, with form nested within spawning sites. Deviations from allele frequencies expected under panmixia due to each level of population subdivision were measured using Chakraborty's (1980) GDA model:

$$H_T = H_W + H_Y + H_F + H_S + H_L + H_D$$

where H_T is the total diversity among and within sites, H_W is the diversity within single samples, H_Y is the diversity among years within sites, H_F is the diversity between sockeye and kokanee within sites, H_G is the diversity among spawning sites within lakes, H_L is the diversity among lakes within drainages, and H_D is the diversity among drainages.

Results

Annual Variation in Allele Frequencies

Five of the loci assayed were polymorphic in some of the kokanee and sockeye populations examined (Table 1). These were *Pgm-1*, *Pgm-2*, *Ldh-4*, *Alat*, and *Gl-2*. The observed genotype frequencies did not differ significantly ($P > 0.05$) from those expected under Hardy–Weinberg equilibrium in any of the populations sampled. In addition, there were no significant differences in genotype frequencies over years at any of the individual loci examined, or for all loci combined, for the three kokanee (Takla Lake; Pierre Creek, Babine Lake; Lower Shuswap River) and two sockeye (Takla Lake; Pierre Creek, Babine Lake) populations sampled repeatedly (Table 1). This suggests that the genetic structure of *O. nerka* populations is stable, at least over relatively short time periods. Accordingly, the electrophoretic information within populations was pooled over years for most subsequent comparisons among populations.

Comparison of Sympatric Sockeye and Kokanee in Three Lake Systems

Sockeye from these streams in Babine Lake where sockeye spawn sympatrically with kokanee (Pierre, Twain, and Tachek creeks) and from two larger systems where spawning kokanee are largely absent (Fulton River and Pinkut Creek) showed no evidence of genetic differentiation at any of the three polymorphic loci examined ($P > 0.05$). Similarly, there was no evidence of differentiation among the kokanee populations at any of the three to five polymorphic loci examined ($P > 0.05$). In contrast, there were significant differences between sockeye and kokanee in all systems where they spawn sympatrically ($P < 0.05$). In Twain and Tachek creeks, sockeye and kokanee differed significantly in genotype frequencies at *Pgm-2* and *Ldh-4* whereas, in Pierre Creek, differentiation was significant at only *Ldh-4* (although the direction of difference at *Pgm-2* was similar to that observed in the others). Thus, there were high similarities within forms and consistent differences between forms across the Babine Lake spawning localities sampled (Fig. 2). Sockeye spawning in the presence of kokanee were no more similar to kokanee than were sockeye spawning in their absence.

Kokanee and sockeye were sampled from three localities in the Shuswap Lake system: the Middle Shuswap River where spawning kokanee are absent, the Lower Shuswap River where the forms spawn sympatrically, and Eagle River where the forms spawn sympatrically but sockeye are rare (and not sampled). Significant differences exist at *Pgm-1* between the Middle and Lower Shuswap River sockeye populations ($P < 0.05$). Similarly, significant differences exist at both *Pgm-1* and *Alat* between the Lower Shuswap River and Eagle River kokanee populations ($P < 0.05$). However, there were greater differences between sockeye and kokanee than between populations within form (Fig. 2). The frequency of *Pgm-1* 100 was consistently higher, and those of *Alat* 91 and 108 consistently lower, in sockeye as compared with kokanee populations in the Shuswap Lake drainage ($P < 0.01$).

Sockeye and kokanee from Narrows Creek, Takla Lake, sampled over 2 and 3 yr, respectively, were consistently different at *Pgm-1* ($P < 0.05$) and *Alat* ($P < 0.01$) (*Alat* was examined only in 1985). Narrows Creek kokanee expressed the *Alat* 91 allele in the highest frequency observed in this study whereas sockeye had the highest observed frequency of *Alat* 100. As a result, genetic differentiation between forms in Takla Lake was greater than that for other sympatric sockeye and kokanee populations (Fig. 2).

In summary, there were significant and consistent genetic differences between sympatrically spawning sockeye salmon and kokanee in all systems examined. Within systems, the extent of genetic differentiation within forms was always less

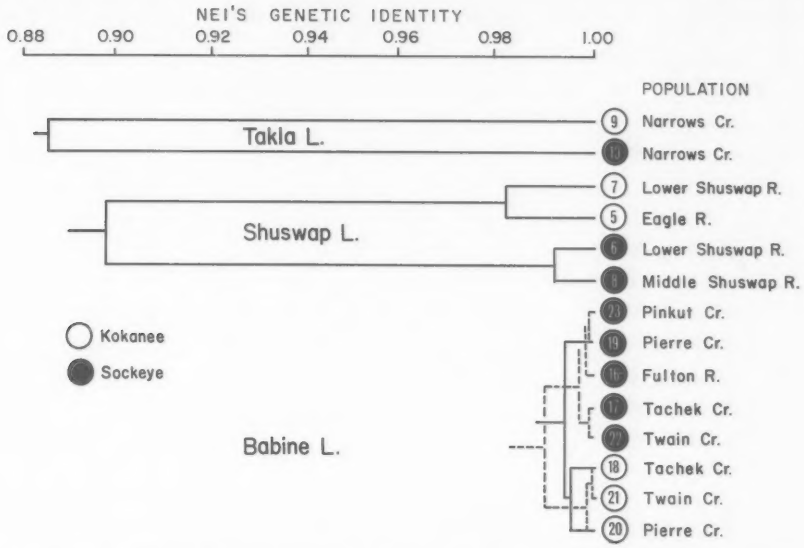

FIG. 2. Phenograms based on measurements of genetic similarity for each of the systems examined where sockeye and kokanee spawn sympatrically (Babine Lake, Shuswap Lake, and Takla Lake). Phenograms based on data taken from five polymorphic loci (all lakes) are drawn in solid lines; those based on three polymorphic loci (Babine Lake) are drawn in broken lines.

than that observed between forms, but the extent of this differentiation varied greatly between lakes. While sympatric sockeye and kokanee differed consistently in allele frequencies, they shared the same alleles at the loci examined. This indicates greater similarity between sympatric forms than that suggested through the use of Nei's genetic identity, which compares only differences in allele frequencies and not the presence or absence of alleles. For example, all sockeye and kokanee populations sampled from the Shuswap Lake system expressed the *Alat* 108 allele, which was largely absent from all other localities (Table 1). Similarly, all sockeye and kokanee populations sampled from Babine Lake expressed the *Ldh-4* 85 allele, also largely absent outside this system (Table 1; Withler 1985). Takla Lake sockeye and kokanee expressed the same allele types at the loci examined, although they displayed none unique to the system.

Overall Comparison of Sockeye and Kokanee in British Columbia

Oncorhynchus nerka populations from across British Columbia cluster into two broad groups (Fig. 3). One cluster includes all sockeye and kokanee populations sampled from the upper Fraser and Columbia River watersheds (with the exception of Skaha Lake and Lower Shuswap River kokanee). The other cluster includes the sockeye and kokanee populations of Babine Lake (Skeena River system). The three coastal *O. nerka* populations examined are split between the two clusters, with Cultus Lake sockeye clustering closely with Babine Lake sockeye and Cowichan Lake kokanee, and Cheewhat Lake sockeye clustering with upper Fraser and Columbia River *O. nerka* populations. There is no obvious separation among populations based on form (sockeye or kokanee). The broad grouping of Columbia and Upper Fraser River *O. nerka* populations, coupled with the separation of the Skeena River populations (Babine Lake) and the splitting of coastal populations between the groups, is very similar to that reported by Utter et al. (1984) in their extensive geographic examination of sockeye salmon at 50 loci.

The genetic relationship of sockeye and kokanee was investigated using ANOVA on allele frequencies at three loci. There were no significant differences between forms in allele frequencies at *Pgm-1*, *Pgm-2*, or *Ldh-4* alleles (Table 3). Differences among drainage systems and the interaction of drainage system and form accounted for most of the observed variance in allele frequencies. These results indicate that it is not possible to classify *O. nerka* populations as sockeye or kokanee solely from knowledge of the allele frequencies at the loci assayed, but classification might be possible given knowledge of the drainage system sampled. These conclusions are supported by the more limited data for *Alat* and *Gl-2*, which also displayed no consistent differences between sockeye and kokanee.

Gene diversity analysis (Nei 1973) yielded similar results. On average, over 90% of the variation in allele frequencies at the three polymorphic loci assayed was due to variation within populations (Fig. 4). Differences among drainage systems were consistently the second greatest source of variation at each locus, followed usually by differences among lakes, between forms, and among years (Table 4).

Discussion

Sockeye and kokanee occur naturally in Japan, Kamchatka, Alaska, British Columbia (including Vancouver Island), and the northwestern United States (Nelson 1968a; Ricker 1972; Scott and Crossman 1973). It seems likely that sockeye originally colonized these diverse regions and subsequently gave rise to nonanadromous populations (kokanee) (Ricker 1940; Nelson 1968a).

The present study supports these conclusions regarding the polyphyletic relationship between sockeye salmon and koka-

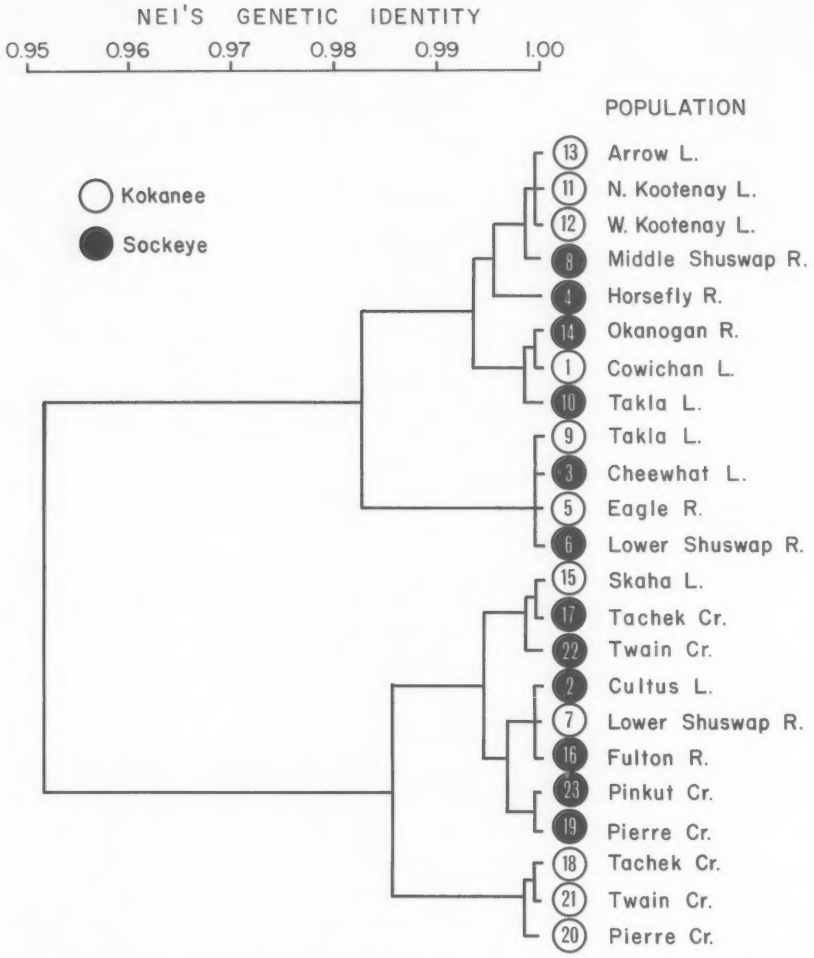

FIG. 3. Phenograms of the relationship among sockeye salmon and kokanee populations based on measurements of genetic similarity at three polymorphic loci.

TABLE 3. Results of a mixed-model ANOVA to determine the sources of variation in allele frequencies at three polymorhic loci. Percent of total variation and $F_{1,1}$ statistics are presented for the random effects and fixed effect, respectively.

	% of total variation				
Source	*Pgm-1* 100	*Pgm-2* 100	*Ldh-4* 100	*Ldh-4* 115	*Ldh-4* 85
Random effects					
error (among years)	5.31	18.26	1.87	8.91	2.35
Sites	12.67	0.00	1.22	46.55	0.56
Lakes	25.14	0.00	0.00	1.55	0.00
Drainage	17.05	38.14	29.35	0.00	14.96
Form – drainge	39.84	43.60	67.56	42.99	82.13
Fixed effect					
Form	0.78	0.50	1.09	0.68	1.04
	$P > 0.25$	$P > 0.25$	$P > 0.25$	$P > 0.25$	$P > 0.25$

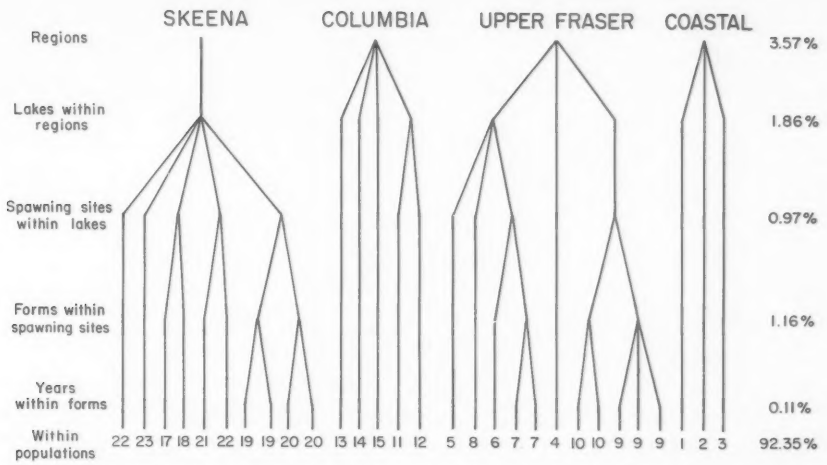

FIG. 4. Hierarchy used in gene diversity analysis of sockeye salmon and kokanee. Percentages at the far right are the average proportions over three loci of total genetic variation occurring at different levels. Population numbers correspond to those in Table 1.

TABLE 4. Percentage distribution of electrophorectically detectable gene diversity at three polymorphic loci among 29 collections of sockeye salmon and kokanee.

Source of variation	Relative gene diversity			
	Pgm-1	*Pgm-2*	*Ldh-4*	Mean
Within populations	89.35	96.10	96.13	92.35
Among years	0.12	0.09	0.02	0.11
Among forms	1.38	0.81	1.16	1.16
Among sites	1.55	0.20	0.44	0.97
Among lakes	3.07	0.40	0.04	1.86
Among drainages	4.52	2.39	2.22	3.57

nee. There were no consistent electrophoretic differences between sockeye and kokanee in British Columbia by which the forms could be separated. There were greater differences among *O. nerka* populations among drainages than there were between sockeye and kokanee. Therefore, it seems unlikely that the existing sockeye and kokanee populations in British Columbia are derived from separate genetic lineages.

The anadromous and nonanadromous forms of other salmonids also appear to have given rise to one another on numerous occasions throughout their range. Nordeng (1983) demonstrated that both anadromous and nonanadromous forms of Arctic char (*Salvelinus alpinus*) can produce anadromous and nonanadromous progeny, the propensity to do so depending on parental form and environmental conditions. Johnson (1980) compared the distribution of anadromous and nonanadromous stocks of Arctic char and concluded that one form gave rise to the other on numerous occasions throughout the species' range. Similarly, Osinov (1984) concluded that anadromous and nonanadromous forms of brown trout (*Salmo trutta*) in the U.S.S.R. were "interchangeable." Supporting this, Ryman (1983) demonstrated that anadromous and nonanadromous brown trout from Sweden were not members of distinct genetic lineages. Allendorf (1975) and Wilson et al. (1985) found that anadromous (steelhead) and nonanadromous rainbow trout (*Salmo gairdneri*) populations were genetically interrelated. McGlade and MacCrimmon (1979) showed that anadromous and nonanadromous brook trout (*Salvelinus fontinalis*) were genetically interrelated. Stähl (1987) demonstrated that nonanadromous and anadromous Atlantic salmon (*Salmo salar*) within broad geographic regions were genetically more similar to each other than they were to their respective forms between regions.

Genetic differentiation among local populations has been documented on numerous occasions in a variety of salmonid species (e.g. Vernon 1957; Frost 1965; Ryman et al. 1979; Ferguson and Mason 1981; Parkinson 1984; Osinov 1984; Wilmot and Burgner 1985; Crozier and Ferguson 1986; Hindar et al. 1986; Campton and Utter 1987). Such differentiation is commonly attributed to the virtual isolation among populations which results from accurate homing to separate spawning grounds (philopatry) (e.g. Vernon 1957; Hartman and Raleigh 1964; Frost 1965; Behnke 1972; Ryman et al. 1979; Crozier and Ferguson 1986; Quinn et al. 1987) or from temporal isolation within spawning grounds (Aspinwall 1974), coupled with the effects of genetic drift (Allendorf and Phelps 1981; Campton and Utter 1987). However, genetic differences between sympatric sockeye and kokanee are difficult to explain solely on these grounds. Genetic differences exist between the forms even where they spawn predominantly in the same place at the same time, indicating that isolation through homing to separate spawning areas cannot account for observed differences between forms. Because spawning populations of *O. nerka* at our study sites were usually large (>1000 to >500 000 individuals), differences between forms are not likely to have arisen from recent genetic drift, although historical bottleneck effects cannot be ruled out. If the genetic heterogeneity observed between sympatric sockeye and kokanee was solely the product of genetic drift (see Allendorf and Phelps 1981), we would expect to see significant heterogeneity within forms as well as between forms within lakes. This was not the case in our extensive survey of spawning populations in Babine Lake. There were no significant genetic differences among either the five separate sockeye spawning populations examined or among the three kokanee populations examined. Furthermore, it is improbable that the observed differences are due to sampling

error or yearly variation, as our sample sizes were large and replicated.

It is not yet clear how genetic differences between sympatrically spawning sockeye and kokanee originate or how they are maintained. McCart (1970) concluded that sockeye and kokanee spawning in the small tributaries to Babine Lake were part of the same panmictic population. He noted that sockeye and kokanee intermixed on the spawning grounds and that kokanee males (like jack sockeye males) showed a strong propensity to act as "sneaks" to spawning pairs, suggesting that significant interbreeding occurred between the forms. McCart (1970) demonstrated that kokanee males fertilized the eggs of sockeye females in the absence of competition and showed that the progeny of such crosses were fully viable in hatchery conditions. However, the present electrophoretic comparison of sockeye and kokanee clearly demonstrates genetic differentiation between forms in Babine Lake tributaries. Despite apparent interbreeding, there is an effective restriction in gene flow between sockeye and kokanee that indicates that they do not constitute a single panmictic population.

The observed genetic similarity and sharing of alleles between populations of sockeye and kokanee in Babine, Takla, and Shuswap lakes strongly indicates that, in each case, one form has arisen from the other in sympatry. The plausibility of sympatric divergence is supported by knowledge of the biology of *O. nerka*. Native and introduced populations of sockeye salmon are known to have given rise to nonanadromous individuals (Ricker 1938, 1940, 1959, 1972; Smirnov 1959; Krokhin 1967; Krogius 1981) and in some cases self-sustaining populations (Ricker 1959; Scott 1984). Similarly, kokanee can give rise to sockeye (Foerster 1947). However, there is no conclusive evidence that the genetic similarities observed between forms within lakes are the result of sympatric divergence. Other mechanisms can be proposed to account for the patterns of similarity observed. The forms may have evolved in allopatry and subsequently introgressed and/or have been subjected to convergent selection pressures in sympatry. However, neither of these alternative hypotheses appears to be tenable. If introgression between the forms had occurred, one would expect to find greater genetic similarity between sockeye and kokanee where they spawn sympatrically than where they spawn in separate localities within the same lake because of the opportunity for continuing introgression. The extensive comparison of Babine Lake sockeye and kokanee spawning populations provides no evidence of such continuing introgression. Sockeye that spawn in the absence of kokanee in Fulton River and Pinkut Creek were genetically indistinguishable from sockeye that spawn sympatrically with kokanee in Pierre, Twain, and Tachek creeks. Similarly, in the Shuswap lake system, sockeye that spawn sympatrically with kokanee in the Lower Shuswap River are genetically no more similar to kokanee than sockeye that spawn in the absence of kokanee in the Middle River system.

It seems even more improbable that convergent selection pressures within lakes could account for the observed genetic similarity of sympatric sockeye and kokanee, since alleles at the loci examined are generally considered to be neutral or nearly neutral to selection (e.g. Ryman 1983). Alternatively, if these alleles are not selectively neutral, and convergent selection pressures were responsible for the observed genetic similarity, one might expect *O. nerka* populations in neighbouring lakes to be genetically similar as well. Babine Lake, in the Skeena drainage, and Takla Lake, in the Fraser Drainage, are separated by a distance of less than 100 km and are morphometrically similar; they occur in the same climatic zone and have similar geological and colonization histories and fish species compositions (Lindsey and McPhail 1986). The physical and biotic similarities between lakes should give rise to similar selective regimes, and in the absence of founder effects, sockeye and kokanee in the two lakes might be expected to be genetically and phenotypically similar. In fact, very substantial differences were found both in allele frequencies and allele types among sockeye and kokanee populations in Takla and Babine lakes. Moreover, sympatric sockeye and kokanee in Takla Lake show greater differences in allele frequencies and gillraker counts (Nelson 1968b) than do those in Babine Lake. These results suggest that lacustrine selection pressures alone do not account for the genetic similarity observed between sympatric forms.

This study, together with previous studies of anadromous and nonanadromous *O. nerka* occurring in sympatry, suggests that genetically isolated populations of sockeye and kokanee have arisen in sympatry, probably on numerous occasions throughout the species' range. The sympatric origin of different forms followed by genetic divergence is theoretically possible (e.g. Maynard Smith 1966; Rosenzweig 1978; Pimm 1979; Rice 1984) but its occurrence in nature is extremely difficult to substantiate and is widely disputed (Mayr 1970; Futuyma and Mayer 1980; Templeton 1981). The biology of *O. nerka* meets two critical requirements of models for sympatric speciation. First, the selective pressures experienced by anadromous and nonanadromous individuals are probably very different and would be expected to promote the genetic divergence of the forms (Ricker 1940). Second, rather strict assortative mating by form would occur within a single generation of the sympatric origin of the forms because of the great size difference between them at maturity. Even within forms, assortative mating by size is conspicuous in wild populations (Hanson and Smith 1967; Foote 1988). Strong assortative mating greatly facilitates genetic differentiation caused by selection (Maynard Smith 1966). Once differences of selective value have accrued, "hybrids" would likely have lower fitness than either "pure" sockeye or kokanee. Such selection can lead to the evolution of premating ethological isolating mechanisms (Maynard Smith 1966), which may partially account for the premating isolation observed between sockeye and kokanee in Babine Lake tributaries (Foote and Larkin 1988). In Pierre Creek, kokanee males prefer to mate with kokanee females rather than with the larger, and more fecund, sockeye females.

In conclusion, recent work describing ecological, morphological, and biochemical differentiation of sympatric forms in salmonids suggests that divergence in sympatry is possible and may be widespread (Johnson 1980; Savvaitova 1980; Balon 1980; Balon and Penczak 1980; Hindar et al. 1986). This study provides further evidence to support this hypothesis.

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Disposition and Elimination Routes of 17α-Methyltestosterone in Rainbow Trout (*Salmo gairdneri*)

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The excretion routes and tissue distribution of [^{14}C]17α-methyltestosterone (17MT) were investigated in rainbow trout (*Salmo gairdneri*) after a single intragastric dose (40 μg, 126.8 kBq); about 95% of the ingested 17MT was absorbed. Total excretion 24 h after treatment accounted for 67.4% of the dose and most of this radioactivity was eliminated into the aquarium water, probably through the gills. Fecal and urinary ^{14}C accounted for 22 and 0.5%, respectively, of the excreted radioactivity. Three days after dosing, 23% of the ingested radioactivity was stored in the whole body and part of the residue (about 20%) was bound to tissues. No detectable amounts of unchanged 17MT were found in tissues 48 h after dosing. In liver, 17MT-derived radioactivity was present primarily as glucuronide conjugates whereas in muscle, radioactivity originated essentially from free metabolites. Maximum bile ^{14}C concentrations were from 200 to 2000 times the levels found in other tissues. This radioactivity was associated with glucuronide conjugates only. Excretion of 17MT-derived radioactivity occurred primarily as free metabolites in aquarium water and as glucuronide conjugates in feces. These results demonstrate the extent of biotransformation of 17MT in trout and suggest the occurrence of several metabolic pathways for this steroid in fish.

Les auteurs ont étudié les voies d'excrétion et la répartition dans les tissus de la truite arc-en-ciel (*Salmo gairdneri*) de la 17α-méthyltestostérone marquée au ^{14}C administrée à raison d'une dose intragastrique unique (40 μg, 126,8 KBq) dont 95 % environ était absorbée. L'excrétion totale après 24 h représentait 67,4 % de la dose et la plus grande partie du carbone radioactif était éliminé dans l'eau de l'aquarium, probablement à travers les branchies. Le niveau de radioactivité du ^{14}C dans les matières fécales et l'urine représentait, respectivement, 22 % et 0,5 % du carbone radioactif excrété. Trois jours après l'administration de la dose, 23 % du carbone radioactif ingéré se retrouvait dans l'organisme et une partie des résidus (20 % environ) était liée aux tissus. Aucune quantité décelable de 17MT non modifiée n'a été retrouvée dans les tissus après 48 h. Dans le foie, la radioactivité associée au 17MT se retrouvait surtout dans les conjugués glucuronides tandis que dans les muscles elle était essentiellement liée aux métabolites libres. Les concentrations maximales de ^{14}C dans la bile étaient de 200 à 2000 fois supérieures à celles des autres tissus. Cette radioactivité était totalement associée aux conjugués glucuronides. L'excrétion de la radioactivité ayant pour origine le 17MT se produisait surtout sous la forme de métabolites libres, dans l'eau de l'aquarium, et de conjugués glucuronides, dans les matières fécales. Ces résultats montrent l'étendue de la transformation biologique de la 17MT chez la truite et porte à croire à l'existence de plusieurs voies métaboliques pour ce stéroïde.

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Sexual maturation of rainbow trout (*Salmo gairdneri*) can be accompanied by deleterious changes that lower the value of cultured trout. Males, which usually mature 1 yr before females, have retarded growth, bad feed conversion efficiency, and are very susceptible to disease.

As discussed by Johnstone et al. (1978), Donaldson and Hunter (1982), and Chevassus et al. (1984) the two main options for avoiding this economic loss are either to produce stocks which are all female or to create sterile fish. Depending on the dose used, oral administration of 17α-methyltestosterone (17MT) has been reported to produce masculinization (as a first step in the production of all-female stocks) or sterility of rainbow trout (Hunter and Donaldson 1983; Solar et al. 1984). Additionally, the use of 17MT as a growth promoter in salmonids has also been investigated (Fagerlund and McBride 1975; Yamazaki 1976; Fagerlund et al. 1979) and a pronounced increase in growth rate has been observed in treated fish.

Based on these results, it is apparent that 17MT has an important economic potential for use in fish culture. However, before this synthetic steroid may be used on a large scale, it is necessary to investigate its fate in treated fish and to measure the quantity of 17MT and/or metabolites released into the environment.

Although some studies describe the depletion rates of orally administered [^{3}H]17MT on the basis of residual levels of radioactivity in tissues (Fagerlund and Dye 1979; Johnstone et al. 1983; Goudie et al. 1986a, 1986b), there are no published reports dealing with the tissue distribution and the elimination routes of both parent compound and metabolites of 17MT in fish. We provide such data by determining the metabolic balance and kinetics of residues for unchanged 17MT and its metabolites.

Materials and Methods

Radiochemicals

^{14}C-labeled 17MT was prepared by the Grignard reaction of [4-^{14}C]dehydroepiandrosterone (purchased from Amersham,

England) with methylmagnesium iodide according to the procedure described by Hyde et al. (1954a), followed by the Oppenauer oxidation with aluminium isopropoxide in which acetone was replaced by cyclohexanone as hydrogen acceptor, as reported by Hyde et al. (1954b). [4-^{14}C]17MT was purified by thin-layer chromatography (TLC) using silica gel plates (Kieselgel 60, Merck) and chloroform–ethylacetate (4:1, v/v) as the solvent. The specific activity of the final product was 1.91 GBq/mmol with a radiochemical purity, as evaluated by radio-TLC and radio-high-performance liquid chromatography (HPLC) of 96%. [4-^{14}C]17MT was diluted with nonradioactive 17MT (Sigma) to a final specific activity of 3.17 MBq/mg, prior to preparation of the medicated feed.

Animal Treatments

Immature rainbow trout (mean weight 140 g) were obtained from the Institut National de la Recherche Agronomique pisciculture at Donzacq (Landes, France) and held in flowing dechlorinated tap water at 16°C for at least 1 wk before use. The pH was constant at 7.3 and the photoperiod was 12 h light (0600–1800) and 12 h dark. Once a day the animals were fed *ad libitum* a pelleted commercial fish diet (Trouvit, France). Fish were starved 48 h before each experiment. At the beginning of the experiment, 15 fish were anesthetized in a 0.05% (v/v) solution of 2-phenoxyethanol (Kock-light Lab. Ltd) and force-fed a gelatin capsule containing 1.60 g of feed and 40 μg of [4-^{14}C]17MT (126.8 kBq).

Sample Handling

The urinary duct was cannulated as described by Cravedi and Tulliez (1981) and each fish was placed in a 5-L well-aerated aquarium. Urine was collected continuously in test tubes that were changed every 8 h. The collected samples were kept frozen (−20°C) until analysis. During the first 24 h, aquarium water was not renewed and fecal pellets were removed by suction using a pipe connected to a rubber bulb pump immediately after being voided into the pond; aquaria were then supplied with freshwater (4 L/min).

Groups of three animals were killed by cervical dislocation after 8, 16, 24, 48, and 72 h. After sacrifice, the aquarium

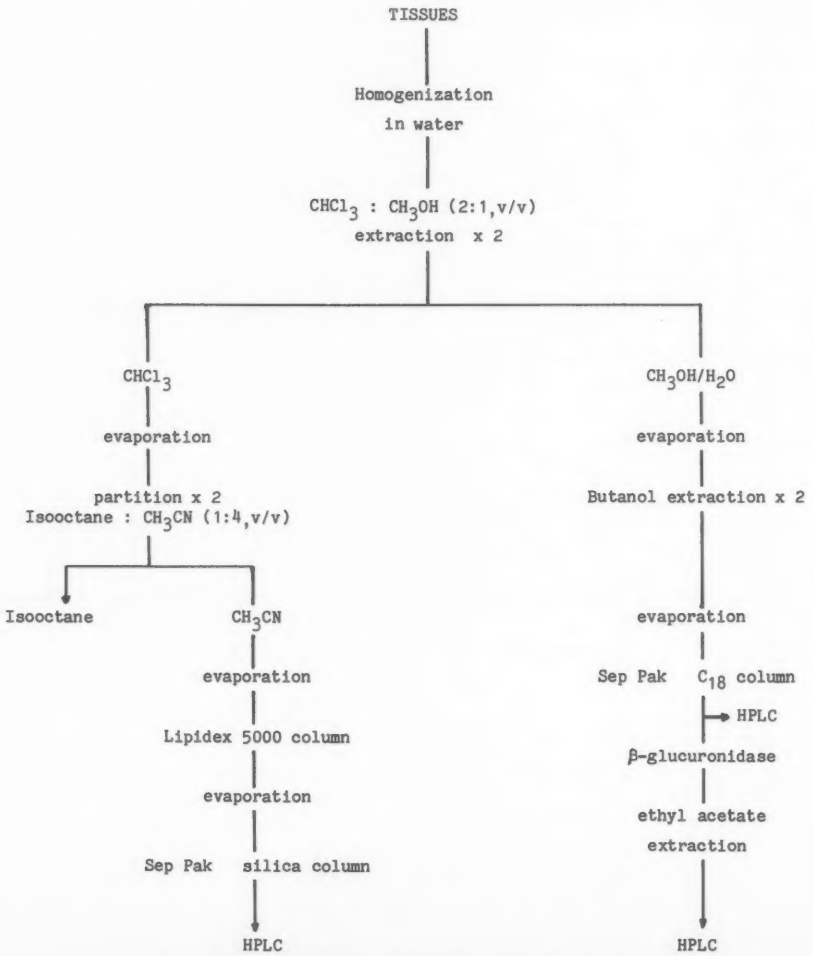

FIG. 1. Flow diagram for isolation of tissue and fecal metabolites.

water was freeze-dried and the residue redissolved in 100 mL of water and kept at −20°C until analysis. The fish were wiped and weighed. Approximately 0.5 mL of blood was collected by intracardiac puncture into a 1-mL heparinized syringe and deposited into oxidizer capsules for radioactivity measurements. The gall bladder was excised and the recovered bile was combined with 0.5 mL of ethanol. Aliquots were placed in capped scintillation vials for ^{14}C determination. Remaining bile samples were kept frozen (−20°C) for later HPLC profiling. The entire liver and a 10-g piece of latero-dorsal muscle were removed and the remaining carcass was minced using a blender. Radioactivity was measured on aliquots of the homogenized tissues and the remaining samples were analysed for [^{14}C]17MT and [^{14}C]17MT metabolites by HPLC.

Radioactivity Measurements

Aliquots of urine, bile, and aquarium water were counted directly in a Packard Tricarb 4430 liquid scintillation counter by using an ACS II (Amersham) fluor mixture. Total radioactivity of tissues, organs, blood, and feces was measured after combustion of aliquots (100–400 mg) in an oxidizer (Oxymat, Intertechnique) and trapping of the resulting $^{14}CO_2$ in a scintillation mixture (Carbomax, Lumac).

Analytical Procedure

Figure 1 gives the general procedure for isolation of metabolites from liver, latero-dorsal muscle, remaining carcass, and feces. Tissue samples (1–5 g) were homogenized in 10–50 mL of chloroform–methanol (2:1, v/v) and filtered under vacuum. This step was repeated on the residual material and the extracts combined. Water (0.2 volume) was added to the extracts and the samples were vigorously shaken on a Vortex mixer before centrifugation at 1800 × *g* for 10 min. The aqueous phase was frozen at −20°C and the organic phase was evaporated in vacuo. The residue was taken up with 4 mL of acetonitrile. A partition with 2 mL of isooctane and 4 mL of acetonitrile was performed twice. The pooled acetonitrile fractions were evaporated, dissolved in methanol – water – acetic acid (70:30:0.05, v/v/v), and passed through a column of Lipidex 5000 (Packard Instruments Co., Downers Grove, IL, USA) eluted with 50 mL of the latter solvent mixture, as described by Andersson and Sjövall (1983). The solvents were evaporated and the residue redissolved in 2 × 1 mL of dichloromethane followed by chromatography on Sep Pak silica cartridge (Waters Associares, Milford, MA, USA). Labeled compounds were eluted from the cartridge by 6 mL of dichloromethane–methanol (2:1, v/v), taken to dryness, dissolved in water – methanol – acetonitrile (5:4:3, v/v/v), and kept at −20°C until HPLC analysis.

The aqueous fraction was evaporated in vacuo, dissolved in 5 mL of acidified water (pH 3), and extracted twice with butanol saturated with water. The pooled butanol extracts were evaporated, dissolved in 1 mL of water, and the metabolites extracted with a Sep Pak C_{18} cartridge, as described by Shackleton and Whitney (1980). The solvent was evaporated and the residue dissolved in 1 mL of water. One half of this solution was analyzed by HPLC. The remaining half was treated by β-glucuronidase from *Escherichia coli* (Type VII, Sigma Chemical Co., St. Louis, MO, USA) and extracted by ethyl acetate. Following a second treatment of the aqueous phase with β-glucuronidase and reextraction, the extracts were combined, taken to dryness, and dissolved in 0.2 mL of methanol before HPLC analysis. To monitor recovery after each step, aliquots of the various fractions obtained during this procedure were counted. Analytical procedures for bile, urine, and water were as for tissues, except that the first extraction step was performed with dichloromethane instead of chloroform–methanol.

HPLC Analysis

The HPLC system was composed of the following LKB (Bromma, Sweden) equipment: model 2150 pumps, model 2152 controller, and model 2158 UV detector equipped with 254-nm UV filter. Fractions (150 μL) were collected in disposable 3-mL scintillation counting tubes with a microcollector (model 202, Gilson Medical Electronics, Middleton, WI, USA). After addition of 2.5 mL of the fluor mixture, each fraction was counted in the scintillation spectrometer.

A gradient elution system was developed to resolve the steroid compounds. It consisted of 200 × 4.6 mm ODS 1(5 μm) reversed-phase column protected by a guard column (25 × 3.8 mm inside diameter) packed with ODS 1 (5 μm). The metabolites of 17MT were separated at ambient temperature at a flow rate of 1 mL/min and 9.7 MPa by linear gradient elution as follows: from 0 with 100% A to A/B = 20/80 at 25 min, where A is methanol–acetonitrile–water (40/30/50) and *B* is methanol–acetonitrile–water (40/30/10). The total column recovery of injected radioactivity was checked for each analysis and usually averaged 99%. At the end of the analysis the column was washed with B for 10 min and reequilibrated with the initial mobile phase.

All the solvents used were HPLC or reagent grade. Methanol was from Merck (Darmstadt, Germany) and acetonitrile from Carlo Erba (Milano, Italy). Deionized water was filtered

TABLE 1. Fate of ^{14}C following administration of [^{14}C]17MT to rainbow trout (values are means ± SD from three animals and are expressed as % of administered dose). ND = not determined.

Route of elimination	Time of collection (h)				
	0 – 8	0 – 16	0 – 24	0 – 48	0 – 72
Urine	0.09 ± 0.04	0.17 ± 0.09	0.30 ± 0.05	0.43 ± 0.28	0.51 ± 0.11
Feces	7.78 ± 2.88	13.11 ± 3.33	14.63 ± 3.15	ND	ND
Surrounding water	7.68 ± 1.01	17.12 ± 4.58	52.52 ± 2.81	ND	ND
Total recovery in excreta	15.55 ± 2.34	30.40 ± 4.41	67.45 ± 5.51	—	—
Gut content[a]	39.3	13.7	1.1	0.2	0.1
Whole body	35.93 ± 8.25	46.28 ± 1.01	26.30 ± 1.92	24.59 ± 3.44	23.14 ± 6.14

[a]Pooled samples from three animals.

TABLE 2. Distribution of radioactivity in tissues of rainbow trout treated with [^{14}C]17MT (Bq/g) (values are means ± SD from three animals).

Fresh samples	8 h	16 h	24 h	48 h	72 h
Bile	14 770 ± 2289	26 707 ± 6470	28 064 ± 6140	35 249 ± 10 381	37 333 ± 4714
Liver	1164 ± 615	255 ± 76	307 ± 137	103 ± 22	201 ± 39
Blood	331 ± 103	244 ± 133	115 ± 14	107 ± 7	125 ± 9
Muscle	86 ± 6	83 ± 4	36 ± 5	17 ± 6	18 ± 3
Carcass	525 ± 101	457 ± 11	133 ± 19	106 ± 77	137 ± 51

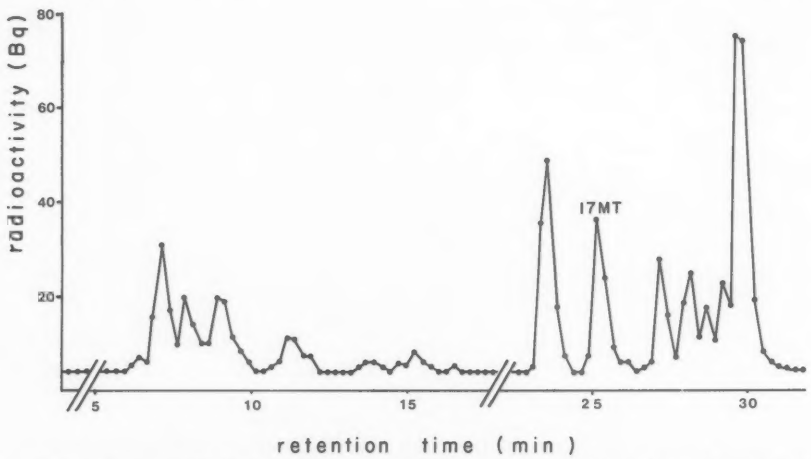

FIG. 2. Reverse-phase HPLC of 17MT metabolites extracted with chloroform from the carcass of [^{14}C]17MT-treated trout. HPLC conditions are described in Materials and Methods.

TABLE 3. 17MT residues in tissues (ng/g) (values are means ± SD from three animals).

Sample	8 h	16 h	24 h	48 h
Liver	9.8 ± 9.4	2.7 ± 1.3	<1	<1
Muscle	5.5 ± 1.3	2.1 ± 1.4	<1	<1
Carcass	52.3 ± 40.5	28.0 ± 9.6	1.5 ± 0.2	<1

through a Millipore HAWP 0.45-μm filter and solvents were filtered through a Millipore FLHP 0.5-μm filter and degassed prior to use.

Identification and Quantification of 17MT

Identification of 17MT on HPLC profiles was established by comparing the retention time with an authentic reference compound (17MT from Sigma Chemical Co, St. Louis, MO, USA). Confirmation by gas chromatography – mass spectrometry (GC–MS) was obtained as follows. The HPLC fractions corresponding to the 17MT retention time were pooled, evaporated in vacuo and dissolved in methanol for analysis in a Hewlett Packard 5992 GC–MS unit (Palo Alto, CA, USA) equipped with a 12.5 m × 0.3 mm OV1 capillary column (Hewlett Packard). Analysis conditions were from 200 to 320°C at 10°/min with a He (carrier gas) flow rate of 1 mL/min. The mass spectrum obtained was compared with that of authentic 17MT. Quantification of 17MT was based on the radioactivity content of the fraction corresponding to the 17MT HPLC peak.

Results

Balance Study and Distribution of ^{14}C in Tissues

A single oral administration of 126.8 kBq of [^{14}C]17MT (40 μg) results in a rapid and important elimination of labeled compounds in the aquarium water, probably via the gills (Table 1). A total of 52.5% of the ingested radioactivity appeared in water after 24 h. Elimination of radioactivity in urine occurred at a very low extent and did not exceed 1% within 3 d. In trout, the maximum radioactvity was observed at 16 h. At this time, 46.3% of the dose was in the whole body. This value, along with the low level of radioactivity in feces, indicates that 17MT was well absorbed in fish. At the end of the 72-h experiment, about 23% of the ingested ^{14}C remained in the whole body.

Maximum ^{14}C concentration occurred at 8 h for all the measurements performed, excepted for bile in which maximum labeling was observed at 72 h (Table 2). It must be noted that bile exhibited the highest concentration values throughout the experiment.

Characterization of [^{14}C]17MT Residues in Tissues

The extraction procedures used (Fig. 1) resulted in radioactivity recoveries of 87.9–94.1%. Reverse-phase HPLC was used to evaluate the chemical nature of the 17MT-derived radioactivity present in extracts of carcass, liver, and skeletal muscle. Under our experimental conditions, the retention time of 17MT, which was entirely extracted in the organic phase, was 25.8 min (Fig. 2).

Only low residues of unmetabolized 17MT were present in liver and muscle, and the depletion rate for 17MT in the carcass was very rapid (Table 3). At 24 h for liver and muscle, and 48 h for carcass, 17MT concentrations were below the detection limit.

In liver, the radioactivity was mainly due to conjugated metabolites (extractable with butanol from the aqueous phase)

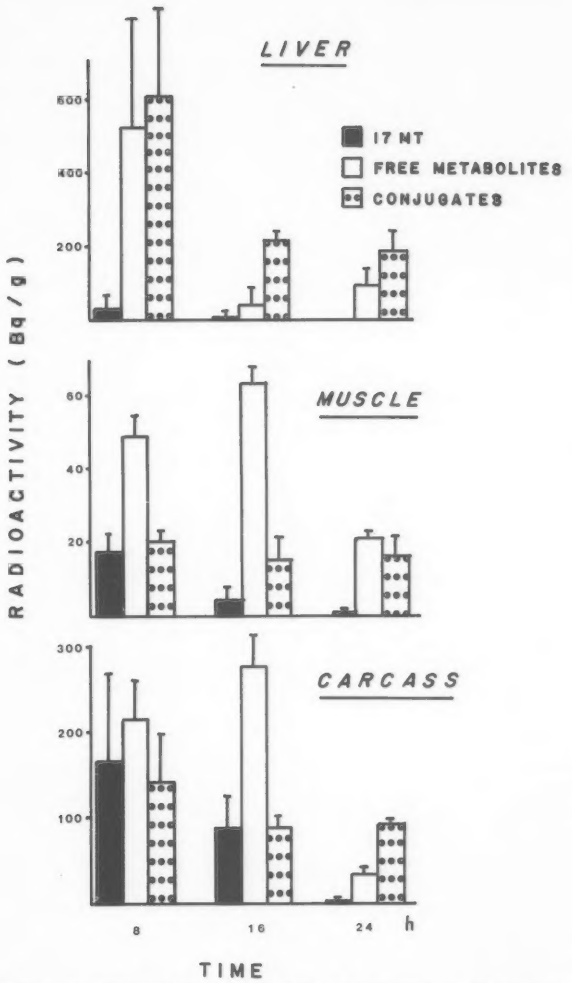

FIG. 3. Evolution and distribution of ^{14}C in liver, muscle, and carcass of trout fed [^{14}C]17MT (126.8 kBq). Values are means ± SD from three animals.

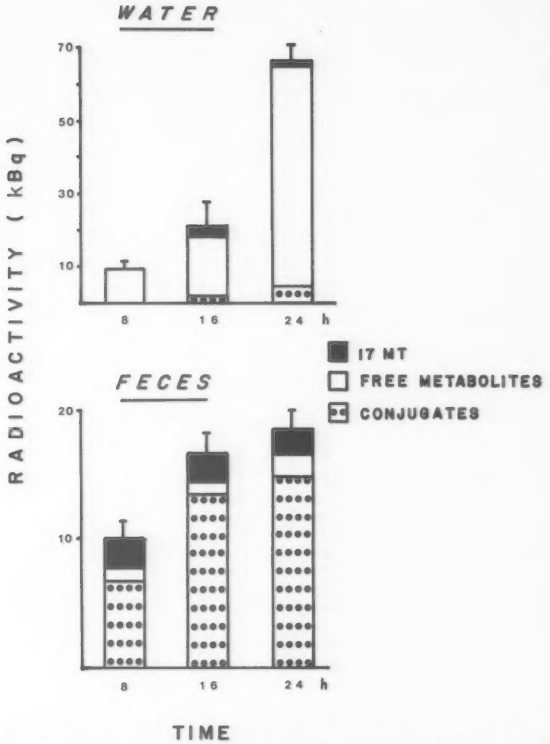

FIG. 4. Evolution and distribution of ^{14}C in excreta from trout fed [^{14}C]17MT (126.8 kBq). For total radioactivity, values are means ± SD from three animals. Proportions of 17MT, free metabolites, and conjugates are from pooled samples.

and to a lesser extent to free metabolites (extractable in chloroform) (Fig. 3). Throughout the experiment, the percentage of ^{14}C due to unchanged 17MT in this organ was less than 3.5%, suggesting a high biotransformation rate.

In muscle and carcass, the predominant radioactive fraction was associated with free metabolites, except for the 24-h carcass samples (Fig. 3). The analysis of radioactivity in 72-h carcasses (data not shown) revealed only background levels of ^{14}C in the organic phase, while 72% of the total labeling was found in the aqueous fraction. After acid hydrolysis of the unextractable material with 6 M HCl under reflux and subsequent extraction with ethyl acetate, about three quarters (75%) of the residual radioactivity was recovered. This material corresponded to labeled compounds with a chromatographic behaviour different from 17MT. No unextractable material was detected in the other samples analyzed.

After β-glucuronidase incubation of the conjugates from liver, muscle, or carcass, all the radioactivity was extracted with ethyl acetate. This treatment completely changed the HPLC elution profile of ^{14}C (data not shown) and resulted in compounds with lower polarity than conjugates, but more polar than 17MT. This suggests that all the radioactivity associated with conjugates fraction corresponds to glucuronide conjugates of one or more 17MT metabolites.

Characterization of 17MT-Derived ^{14}C in Excreta

17MT represents a minor labeled fraction in excreta (Fig. 4). In aquarium water, free metabolites were the most labeled fraction, but in feces, the radioactivity was mainly due to conjugates. Incubation of conjugates isolated from water and feces with β-glucuronidase resulted in the complete hydrolysis of these compounds, indicating that at least one 17MT metabolite may be a glucuronide conjugate.

HPLC analysis of the ^{14}C in bile (top panel, Fig. 5) shows the presence of polar metabolites, but no trace of the parent compound. After enzymatic treatment of the bile with β-glucuronidase (bottom panel, Fig. 5), the peaks between 3 and 8 min are eliminated, while several new peaks are observed eluting later than 8 min. These findings suggest that 17MT metabolites in bile may be glucuronide conjugates.

Considering the weak radioactive material in urine, all the samples were combined for analysis. Conjugates accounted for 69.5% of the radioactivity (results not shown) and free metabolites for 30.5%. No parent compound was detected, and β-glucuronidase treatment of the aqueous phase resulted in complete hydrolysis of urine conjugates.

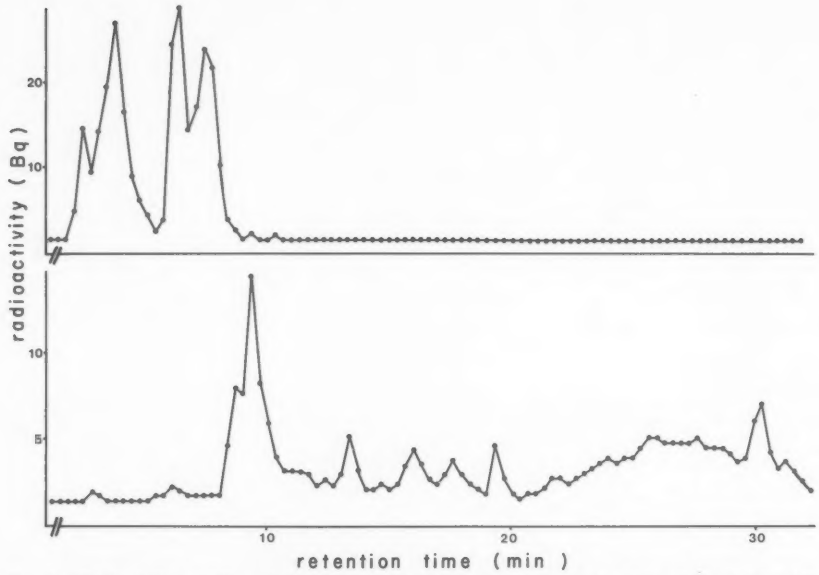

FIG. 5. HPLC elution profiles of [^{14}C]17MT (126.8 kBq). Chromatographs were obtained from bile which was incubated in the absence (top panel) and presence (bottom panel) of β-glucuronidase.

Discussion

To study 17MT residues in fish, it is necessary to use a procedure that enables quantitative extraction of all 17MT-derived substances, in addition to the parent compound. With chloroform–methanol (2:1, v/v), nearly 100% of the radioactivity present in the tissues was extracted. The recovery of labeled steroids through the entire method (i.e. including purification steps) reached 92%. This value exceeds the extraction efficiency usually reported for androgen steroids in tissues (Hoffmann and Rattenberger 1977; Goudie 1984; Anderson and Sjövall 1985). Moreover, in most of these cases, the methods described do not include the extraction and purification of conjugated steroids as evaluated in our work.

Although for some samples, all the present metabolites cannot be completely resolved, the HPLC system we have developed enables the separation between unchanged 17MT and 17MT-derived products to be performed. Therefore, quantification of the administered steroid and its metabolites is made possible, which permits a more detailed study of the fate of 17MT in trout.

The incorporation, distribution, and depletion of total radioactivity are consistent with the findings of several authors. As observed by Fagerlund and Dye (1979) in coho salmon (*Oncorhynchus kisutch*) and by Goudie et al. (1986b) in blue tilapia (*Oreochromis aureus*), the highest levels of radioactivity occurred during the first 16 h, indicating a rapid absorption of the steroid. The high concentration in bile and low level in muscle noted in our study were also reported by these authors.

The depletion of ^{14}C in the whole body was initially rapid, but 3 d after dosing, 23% of the radioactivity ingested was still present in treated fish. This biphasic pattern has been observed in blue tilapia by Goudie et al. (1986b) and could be related to the binding of 17MT and/or metabolites to tissues. In our study, approximately 20% of the ^{14}C remaining in the carcasses 72 h after the radiolabeled diet was fed cannot be extracted, as described in Fig. 1. Only strong acid hydrolysis partly liberates the radiolabeled fraction, suggesting a covalent binding of ^{14}C metabolite(s) to macromolecules. The irreversible binding of steroids to proteins and DNA has been demonstrated by several authors (Brueggemeier et al. 1984; Yu and Fishman 1985; Bucala et al. 1986). In our case, however, because of the limited amount of radioactive material available, no additional characterization was attempted.

The HPLC analysis of the extracted radioactivity demonstrated that almost all ^{14}C was associated with metabolites because no detectable amounts of 17MT were found in 48-h tissue samples. These residues are in the form of glucuronides as well as free metabolites.

The metabolic balance revealed some unexpected results. Surprisingly, less than 1% of the ingested radioactivity was excreted in urine during 72 h. In mammals, including man, treated with 17MT, 20–50% of the dose was usually eliminated via this route (Hyde et al. 1954a; Quincey and Gray 1967; Templeton and Jackson 1983). The minor importance of urinary excretion in trout may be related to preferential elimination across the gill and to the significant role played by the bile in steroid metabolism. For 17MT, approximately 20% of the ingested ^{14}C was eliminated in the gall bladder at 72 h. This elimination occurred after the biotransformation of the parent compound into conjugated metabolites, probably in the liver.

At 24 h, 14.6% of the ingested dose was detected in feces. Fecal ^{14}C may result from (1) unabsorbed 17MT, (2) 17MT and/or metabolites excreted via the bile, or (3) biotransformation products due to the enzymatic activity of the intestinal microflora. This latter possibility does not occur in trout because of the low level of microorganisms in the gut of this animal (Lesel 1981). Because bile did not contain detectable amounts of the parent compound, all the 17MT detected in feces (5.1% dose) resulted from unabsorbed material and therefore, the digestibility of 17MT in *Salmo gairdneri* can be estimated to be nearly 95%. The presence of free metabolites in feces and not in bile

suggests the contamination of feces with 17MT-derived ^{14}C originating from surrounding water in which unconjugated metabolites were predominant.

This study has demonstrated that 17MT concentration in trout tissues decreased rapidly, while detectable amounts of metabolites remained in various tissues and especially in bile. This finding underlines the necessity to include bile in residue control program concerning 17MT and to seek metabolites in addition to the parent compound. The toxicological significance of these metabolites remains to be investigated. In the case of sex-reversal treatment, because the elapsed time between treatment and marketable size of trout, it would be reasonably concluded that no hazardous levels of residues might be ingested by the consumption of hormone-treated fish. However, the characterization of the residues bound to tissues and the demonstration of their safety would be of major concern if 17MT was used as a growth promoter in aquaculture. We are presently attempting to identify the metabolites excreted to evaluate the potential hazard associated with the discharge of steroidal substances in water via the treated fish excreta.

Acknowledgements

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Comparison of Meristic and Morphometric Characters among Spring- and Fall-Spawning Ecotypes of Cisco (*Coregonus artedii*) in Southern Quebec, Canada

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Hénault, M., and R. Fortin. 1989. Comparison of meristic and morphometric characters among spring- and fall-spawning ecotypes of cisco (*Coregonus artedii*) in southern Quebec, Canada. Can. J. Fish. Aquat. Sci. 46: 166–173.

Meristic and morphometric phenotypes of the spring-spawning stock of ciscoes (*Coregonus artedii*) from lac des Écorces were compared with those of fall-spawning stocks from the same drainage basin. The spring spawners show almost complete nonoverlap in gillrakers compared with fall spawners (averages of 42.7 and 50.5, respectively). This large gap could indicate genotypic differences between these stocks. Spring spawners also show smaller numbers of lateral line scales and of dorsal and anal fin rays, which might be related to higher incubation temperatures. Based on the comparison of conventional and truss network measures, discriminant analyses performed separately on males and females showed that the head and cephalic structures are smaller in spring ciscoes; their thoracic region is also longer and their caudal peduncle narrower and shorter. A principal components analysis showed that the few fall-spawning ciscoes captured in lac des Écorces do not differ from the fall spawners occurring upriver: the spring-spawning population would thus be allopatric.

Les phénotypes morphologiques (morphométrie et méristique) du stock de cisco (*Coregonus artedii*) de lac frayant au printemps au lac des Écorces ont été comparés à ceux de stocks du même bassin de drainage frayant l'automne. Il n'y a pratiquement pas de recoupement dans le nombre de branchicténies des ciscos de printemps et d'automne (moyennes de 42,7 et 50,5, respectivement). Ce large écart pourrait indiquer des différences génotypiques entre ces stocks. Les ciscos de printemps ont aussi de plus petits nombres d'écailles de la ligne latérale et de rayons aux nageoires dorsale et anale, ce qui pourrait être relié aux températures d'incubation plus élevées. Des analyses discriminantes menées séparément chez les mâles et les femelles, sur des ensembles de mesures morphométriques conventionnelles et en réseau (« truss network »), ont montré que la tête et les parties anatomiques céphaliques sont plus petites chez les ciscos de printemps; ces derniers ont par ailleurs la région thoracique plus longue, mais le pédoncule caudal plus étroit et plus court. Une analyse en composantes principales a montré que les quelques ciscos d'automne capturés dans le lac des Écorces ne diffèrent pas des stocks d'automne situés en amont : la population frayant au printemps serait donc allopatrique.

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Pariseau et al. (1983) reported the first discovery of a population of spring-spawning cisco (*Coregonus artedii*) from outside the Great Lakes, in lac des Écorces (Labelle County, southern Quebec; Fig. 1). The origin of this population has been the subject of some discussion. One can consider its presence as a relict of the glacial era, the population being located in an area with a high occurrence of glacio-marine species; we could also be witnessing the beginning of a speciation process (Pariseau et al. 1983).

Spring spawning for a cold temperate species such as the cisco implies a site selection suitable for egg development. For the population under study, spawning occurs at a depth of 20–30 m. As incubation temperatures (from 5.5 to 7°C; Hénault 1986) are higher than those normally encountered by fall-spawning ciscoes, we can expect numerous differences in meristic (Hubbs 1926; and others) and morphometric characters, especially in a highly plastic species such as the cisco (e.g. Martin 1949; Svärdson 1979; Todd et al. 1981). Therefore, the main objective of our study is to characterize the meristic and morphometric phenotypes of this unique population and compare them with those of three fall-spawning populations from the same drainage basin, including a few fall-spawning individuals from lac des Écorces. We will discuss our results in view of the possible effects of the delay in the spawning period, and of the ensuing environmental conditions prevailing during embryonic development, on the meristic and morphometric phenotypes of the cisco. We will also look at the status, allopatric or sympatric, that can be assigned to this population.

Materials and Methods

Geographical Location

Lac des Écorces (46°32′N, 75°25′W) is located in the rivière Kiamika basin (Fig. 1), approximately 20 km downstream from Petit lac Kiamika and lac Marquis. The rivière Kiamika is

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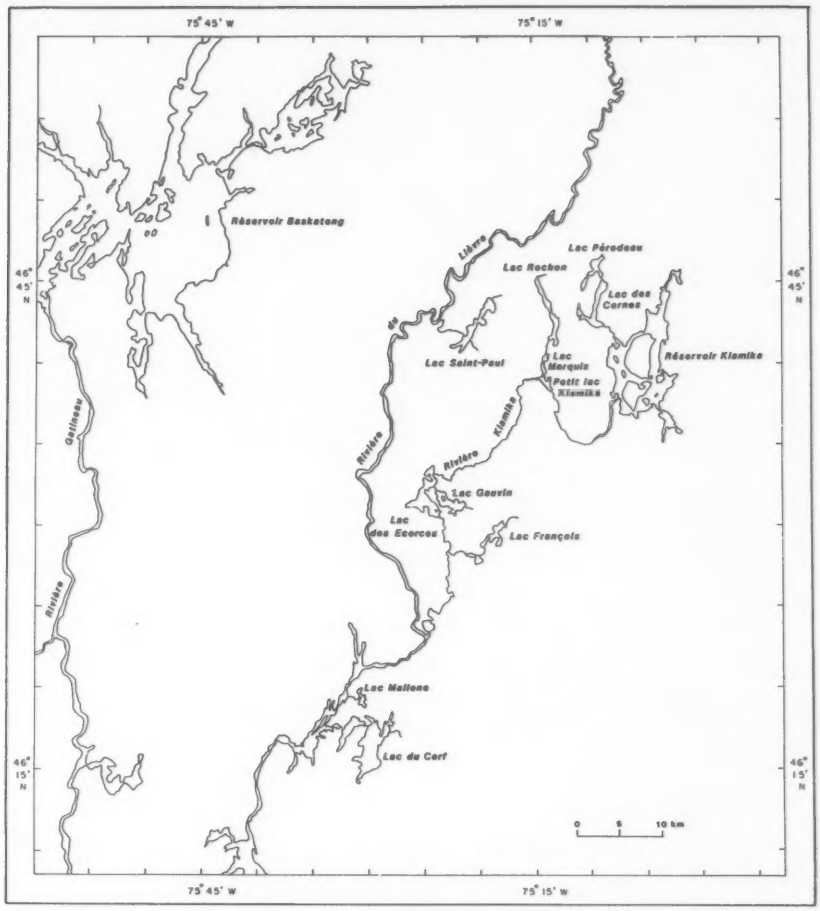

FIG. 1. Study area. Identified lakes and reservoirs were sampled. Except lac des Écorces, none contained spring-spawning ciscoes. Only samples from Petit lac Kiamika and lac Marquis were used in this study.

shallow, about 5 m deep, and could possibly be used as a downstream dispersal route for fish into lac des Écorces. Warm summer water temperatures and the presence of small falls and heavy rapids on this section of the river, however, could block upstream movement of ciscoes and reduce successful downstream movement.

Specimens were sampled in 1983 and 1984 in the three lakes and preserved in formalin before being studied. Spring ciscoes ($n = 910$) were captured exclusively in lac des Écorces whereas fall ciscoes were taken from all three, thus providing four groups of fish for the comparisons.

Examination

Unless otherwise noted, the determination of meristic characters follows the methodology of Hubbs and Lagler (1958). The following characters were enumerated: pored lateral line scales (PSLL) (Lindsey 1962), gillrakers from the lower (LOGR) and upper (UPGR) limbs of the first left branchial arch (Clarke 1973), including rudimentary rakers, dorsal (PDFR), anal (PAFR), and left pelvic (PELV) and pectoral (PECT) principal fin rays, dorsal (RDFR) and anal (RAFR) rudimentary fin rays, and mandibular pores of the right mandible (MAPO).

Among the commonly used conventional measurements, 22 were chosen because of their relevance in the distinction of stocks of ciscoes. Unless otherwise mentioned, the terminology is that of Hubbs and Lagler (1958). The conventional measurements included head length (HEDL) and depth (HEDD, "occiput to isthmus"), length (MAXL; Lindsey 1962) and minimal width of the maxillary (including the supramaxillary; MAXW), mandible length (MANL), snout length (SNTL), eye diameter (EYED; Clarke 1973), pupil diameter (PUPD; Kliewer 1970), interorbital width, (INTW; Clarke 1973), length of the base and height of the dorsal (DOFB, DOFH) and anal (ANFB, ANFH) fins, length of the pelvic (PVFL), pectoral (PCFL), and adipose fins (ADFL; Lindsey 1962), body (BODD) and caudal peduncle (CAPD) depths, predorsal length (PRDL), and total (TL), fork (FL), and standard lengths (SL). The truss system (Strauss and Bookstein 1982) was also used to better characterize general body form (31 measurements; Fig. 2).

The total, fork, and standard lengths were measured to the closest millimetre, while all the other variables were measured with automatic recording calipers (± 0.05 mm) linked to an IBM-PC microcomputer similar to McAllister and Planck (1981). A study of the precision of the various measurements

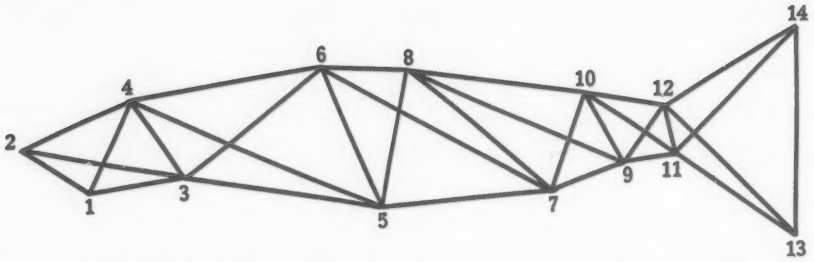

FIG. 2. Locations of 14 landmarks used for constructing the truss network. The 31 distances between these points were measured. Landmarks refer to (1) anterior junction of preopercular and interopercular bones, (2) anterior tip of snout at upper jaw, (3) origin of pectoral fin, (4) most posterior point of neurocranium, i.e. beginning of scaled nape, (5) origin of pelvic fin, (6) origin of dorsal fin, (7) origin of anal fin, (8) posterior insertion of dorsal fin, (9) posterior insertion of anal fin, (10) origin of adipose fin, (11) anterior attachment of the ventral membrane of the caudal fin (not to be mistaken with the depth of the caudal peduncle), (12) anterior attachment of the dorsal membrane of the caudal fin (see No. 11), (13) most posterior-ventral point of naturally extended caudal fin at tip of second caudal ray, and (14) most posterior-dorsal point of naturally extended caudal fin at tip of second caudal ray.

TABLE 1. Meristic counts (means and standard deviations), F values calculated in an analysis of variance of four stocks of ciscoes, and results of Student–Newman–Keuls (SNK) multiple comparison tests.

Variable	Lac des Écorces, spring (n = 45)	Lac des Écorces, fall (n = 10)	Petit lac Kiamika (n = 26)	Lac Marquis (n = 30)	Fall (pooled) (n = 66)	$F_{(3,107)}$[a]	SNK[b]
PECT	15.2 (0.85)	15.3 (0.67)	15.5 (0.71)	15.5 (0.90)	15.4 (0.79)	0.86 NS	(S, F, K, M)
PELV	12.0 (0.50)	11.7 (0.48)	11.5 (0.51)	11.8 (0.43)	11.6 (0.48)	5.53**	(S, F, M) (F, M, K)
RDFR	2.3 (0.45)	2.1 (0.32)	2.1 (0.32)	2.2 (0.43)	2.2 (0.38)	1.03 NS	(S, F, M, K)
PDFR	9.9 (0.68)	11.0 (0.67)	11.0 (0.82)	11.1 (0.58)	11.0 (0.69)	24.92***	(S) (F, M, K)
RAFR	2.0 (0.26)	1.9 (0.32)	2.0 (0.44)	1.9 (0.48)	1.9 (0.44)	0.31 NS	(S, F, M, K)
PAFR	11.0 (0.84)	12.5 (0.53)	12.1 (0.71)	12.2 (0.61)	12.2 (0.64)	24.41***	(S) (F, M, K)
PSLL	71.8 (2.30)	76.5 (3.80)	74.1 (3.60)	75.9 (3.5)	75.3 (3.69)	12.96***	(S) (F, M) (F, K)
MAPO	6.3 (0.81)	6.4 (0.70)	6.1 (0.67)	6.5 (0.82)	6.3 (0.75)	1.05 NS	(S, F, K, M)
LOGR	26.9 (1.14)	32.1 (1.07)[c]	32.1 (1.23)	31.8 (1.02)	32.0 (1.11)[d]	175.9[e] ***	(S) (F, K, M)
UPGR	15.8 (0.78)[f]	18.4 (0.53)[c]	18.4 (1.24)	18.7 (0.98)	18.6 (1.06)[d]	71.9[g] ***	(S) (F, K, M)
TOGR	42.7 (1.65)[f]	50.6 (1.13)[c]	50.5 (2.25)	50.6 (1.71)	50.5 (1.90)[d]	162.2[g] ***	(S) (F, K, M)

[a]NS, nonsignificant ($P > 0.05$); **$P < 0.01$; *** $P < 0.001$.

[b]Nonsignificantly different stocks (P>0.05) are included in the same parentheses: S, lac des Écorces, spring; F, lac des Écorces, fall; M, lac Marquis, K, Petit lac Kiamika.

[c]n = 7.

[d]n = 63.

[e]df = 3,104.

[f]n = 44.

[g]df = 3,103.

led to the elimination of one conventional character (PUPD) and one truss variable (13–14) from the analysis, based on their high coefficient of variation as established from replicate measures taken on the same specimens (Hénault 1986).

Statistical Analysis

ANOVA tests were used to compare means of meristic characters and multiple comparison tests (Student–Newman–Keuls (SNK and Scheffe) were performed where means differed significantly. As the length frequency distribution in our samples is significantly different among the four groups (ANOVA, $P <$ 0.001), we used the allometric transformation (Thorpe 1976) to standardize the data by the common-within group slope, thus eliminating the effects of heterogeneity in size (Reist 1985, 1986; Claytor and MacCrimmon 1987). Discriminant function analyses (SPSS[x] software) were performed on the morphometric data, using the stepwise procedure for the selection of the important variables (method = WILKS). As sexual dimorphism was present within two of the stocks, the sexes were treated separately. As our data did not fulfill the assumption of homoscedasticity, specimens from the three fall-spawning stocks were pooled into one group and discriminant functions were calculated from an equal size random sample of specimens from the spring and fall spawners. Although some information is lost in the process, the real success of classification can then be evaluated for specimens used in the analysis and for the excluded ones.

To examine morphometric relationships between the four groups, the generalized distances of Mahalanobis were calculated between stocks of ciscoes and tested using the F-test (SAS Institute Inc. 1982). Also, a principal components analysis was performed on the variance–covariance matrix of each series of transformed variables. Mean coordinates of each stock, for

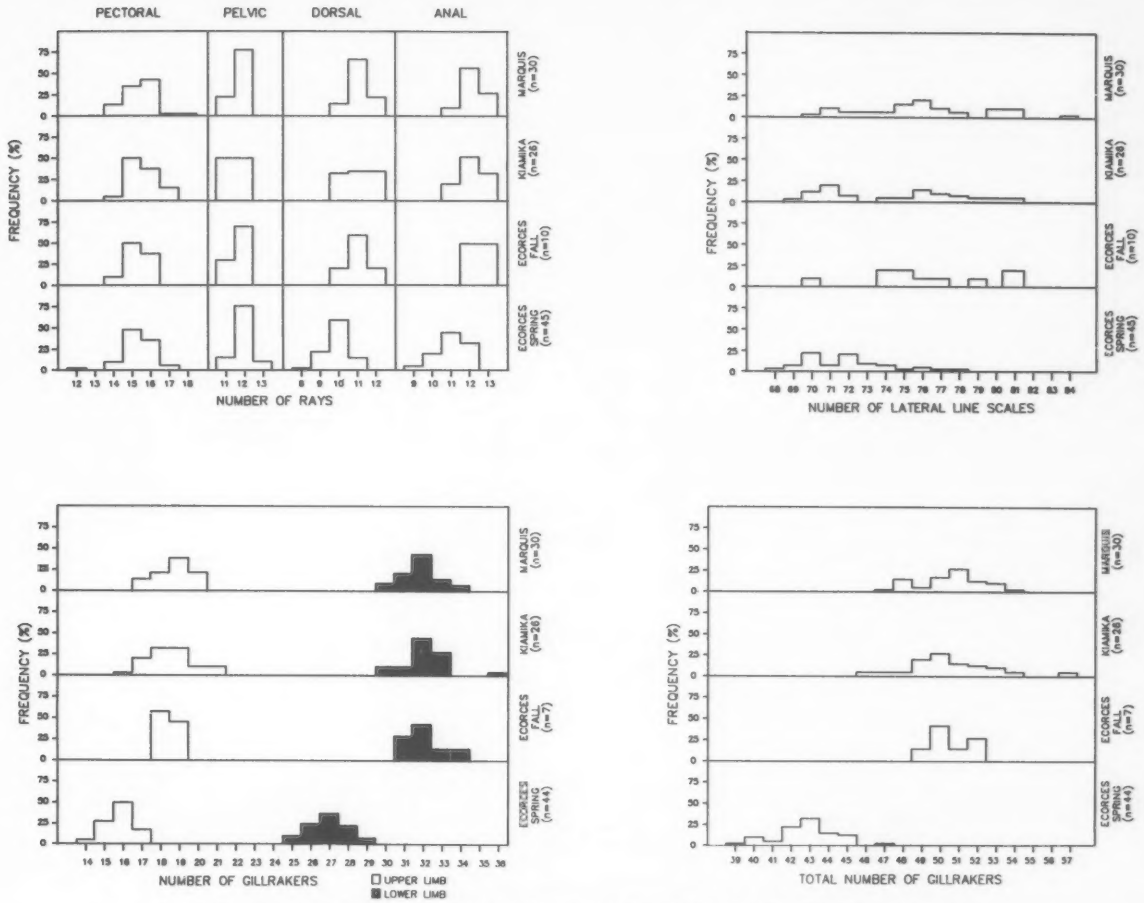

FIG. 3. Frequency distributions of some meristic counts in four stocks of rivière Kiamika basin ciscoes.

principal axes representing more than 5% of the total variance, were compared by ANOVA and Scheffe's multiple comparison test (Pimentel 1979).

Results

ANOVA tests performed on meristic counts show significant differences between the four stocks for 7 of the 11 variables (Table 1). The means for the number of pectoral fin rays, of rudimentary rays of the dorsal and anal fins, and of mandibular pores are not significantly different between the four stocks. Meristic counts do not show significant differences between fall-spawning stocks. Only the number of lateral line scales differs significantly between stocks of Petit lac Kiamika and lac Marquis; Scheffe's test, however, does not show significant differences between the two means. Since the expected number of wrong decisions is higher with the SNK test than with Scheffe's test (Scherrer 1984), we consider these means to be similar.

Means for the number of dorsal and anal fin rays, lateral line scales, and gillrakers are significantly smaller in spring-spawning ciscoes (Table 1; Fig. 3). The greatest difference is observed in the number of gillrakers (average of 42.7 for spring spawners and 50.5 for fall spawners). However, the average number of pelvic fin rays is significantly greater in the spring stock than in the fall stock of Petit lac Kiamika.

Concerning morphometrics, results from the discriminant analyses show significant differences (Wilks lambda, $P < 0.001$) between spring and fall ciscoes of both sexes. For the conventional measures, the canonical correlations are equal to 0.89 for males and 0.92 for females; for the truss system, correlations are 0.90 and 0.94, respectively.

Both systems of measurements (Fig. 4) show the spring males and females to have a relatively smaller head (including generally smaller cephalic parts), as well as a narrower and shorter caudal peduncle, than fall spawners; the predorsal length and the surface of the caudal fin are similar in spring and fall spawners of both sexes. The base of the dorsal and anal fins is shorter in spring male ciscoes whereas their depth and the length of the adipose, pectoral, and pelvic fins show no significant differences between the two groups; these characteristics are the same for the females, with the exception of the dorsal fin height which is smaller in spring spawners. Having a smaller head, spring males and females also show longer 4–6, 3–6, and 3–5 distances (Fig. 4B and 4D). While being in accordance with an equal predorsal length in the two stocks, this shows that the thoracic cell is larger in spring males and females. Finally, body depth is larger in spring females.

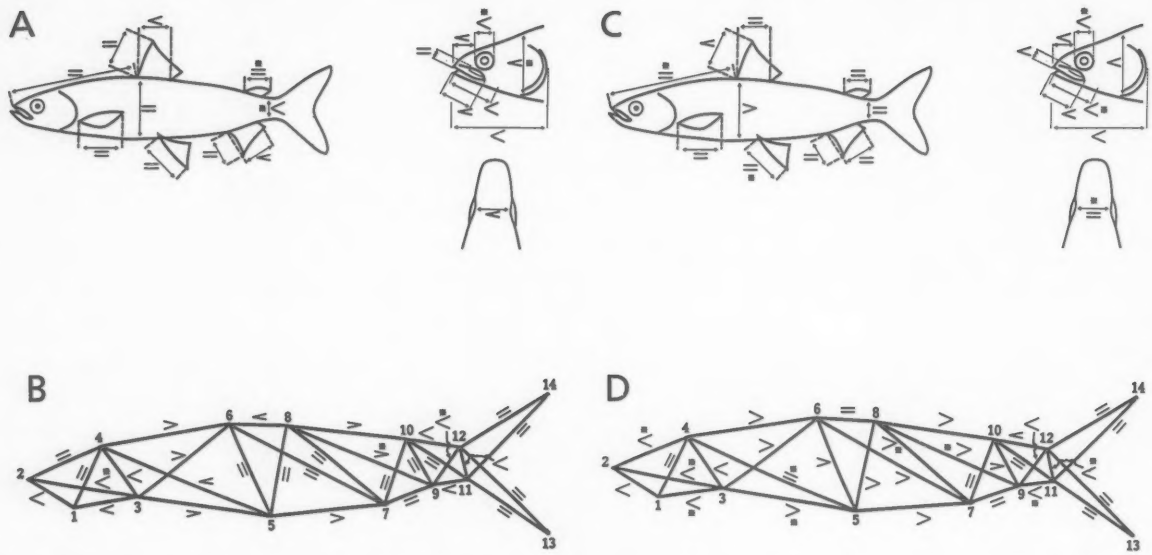

FIG. 4. Significance levels of univariate tests of equality of means for morphometric variables between samples of spring- and fall-spawning ciscoes, and the nature of the difference (=, $P > 0.05$; $< >$, $P < 0.05$; $< >$, $P < 0.01$; $< >$, $P < 0.001$). (A and B) males; (C and D) females. Asterisks indicate variables chosen by the stepwise discriminant analysis.

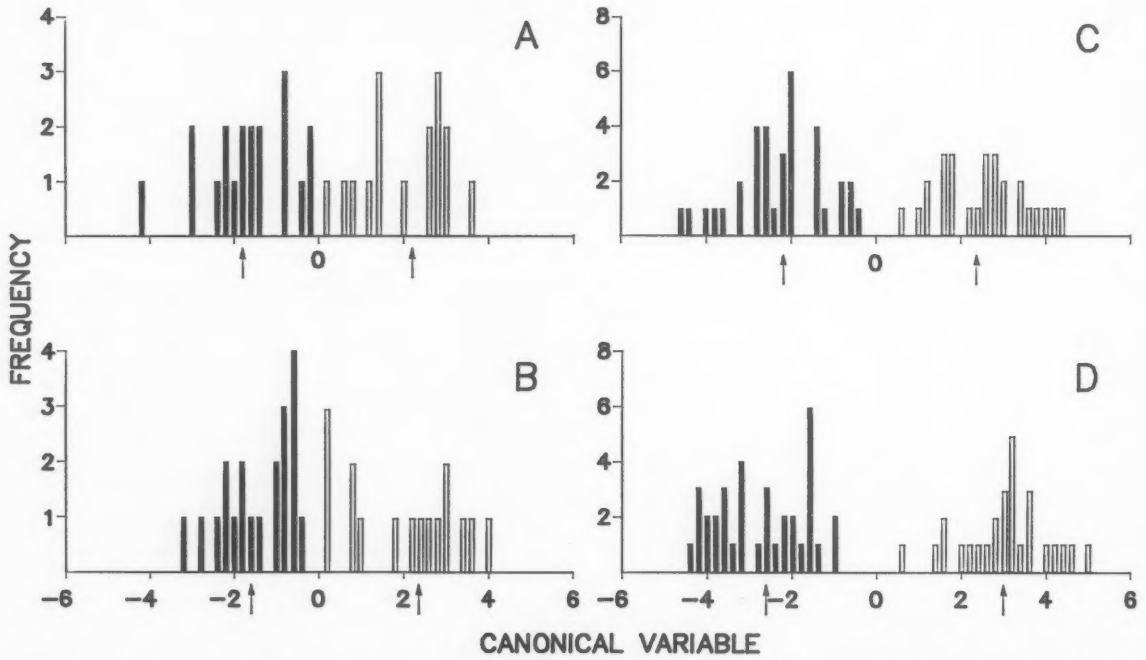

FIG. 5. Frequency distribution of the canonical variable, for a discriminant analysis with (A and C) conventional or (B and D) truss network characters, calculated between spring and fall (A and B) male or (C and D) female ciscoes. Dark bars represent the spring-spawning group and arrows indicate position of centroids.

Based on the classification function involving four conventional variables (Fig. 4A), 31 of the 32 (96.9%) male specimens used in the analysis were correctly assigned to their group of origin and so were the three specimens not used in the analysis. The discriminant function derived from the truss system (three variables; Fig. 4C) produces a 93.7% classification success for the 32 specimens used in the analysis whereas three of the four specimens left out are also correctly classified. Dis-

TABLE 2. Mahalonobis generalized distances between four stocks of ciscoes of the rivière Kiamika basin, estimated with conventional variables (above the diagonal) and with truss network variables (below the diagonal). All distances are highly significant ($P < 0.001$).

Stock	Lac des Écorces, spring ($n = 53$)	Lac des Écorces, fall ($n = 7$)	Petit lac Kiamika ($n = 15$)	Lac Marquis ($n = 31$)
Lac des Écorces, spring ($n = 50$)		3.38	4.34	4.29
Lac des Écorces, fall ($n = 4$)	6.32		2.95	3.57
Petit lac Kiamika ($n = 15$)	7.30	4.83		2.92
Lac Marquis ($n = 36$)	6.07	5.20	4.51	

criminant functions for the conventional and truss network measures include more variables in the females, respectively five and nine (Fig. 4B and 4D). This possibly reflects a greater shape heterogeneity. Both conventional and truss functions, however, correctly classify all female specimens used in the analysis as well as those set aside. This perfect classification is probably due to the greater distance existing between the centroids of the females (Fig. 5).

The generalized distances of Mahalanobis between centroids are greater when calculated with truss network variables than with conventional ones: these are respectively 3.99 and 3.89 for the males and 5.63 and 4.72 for the females. This same observation applies to distances evaluated between the four stocks in a three-dimensional canonical space (Table 2). These distances are all significantly different, which could indicate that the four stocks represent morphologically distinct groups. The largest distances among centroids exist between the spring ciscoes and those of the three fall stocks whereas the smaller distances are those between the stocks from lac Marquis and Petit lac Kiamika and between those two and fall ciscoes of lac des Écorces (Table 2).

Results from the principal components analysis yield a different set of relationships between the groups under study. Lac des Écorces fall spawners do not form any distinct cluster in the reduced space of the first five axes, contrary to the other stocks. This observation is confirmed by Scheffe's test which indicates that the former specimens are not distributed in any significantly different manner from those of Petit lac Kiamika with regard to the first five principal axes whereas all the other intergroup comparisons are significantly different on one or more of the axes (Table 3).

Discussion

The most striking difference among spring- and fall-spawning ciscoes concerns the number of gillrakers which is generally considered to be genetically determined although also influenced by environmental conditions to some degree (Lindsey 1981). Results of this study show a difference of 7.8 elements between spring and fall ciscoes, with averages of 42.7 and 50.5, respectively. This observed difference is greater than what is reported in the literature for sympatric or allopatric pairs of stocks of spring and fall spawners of various species. For example, Svärdson (1979) noted that spring-spawning *Coregonus trybomi* have fewer gillrakers than sympatric *C. albula*, differences within lakes ranging from 0.33 to 3.25. However, a spring-spawning population of Windermere charr (*Salvelinus alpinus*) shows a significantly higher count of gillrakers than the sympatric fall-spawning population, 25.3 and 21.1, respectively (Frost 1965; Ferguson 1981). The observed difference is also greater than the experimentally induced variations in the number of gillrakers reported in the literature. For example, artificial rearing brought a shift in *C. kiyi* gillraker number from 41.5 in the parents to 35.2 in the progeny, a difference of 6.3 elements, while *C. hoyi* and *C. zenethicus* showed no differences (Todd et al. 1981). Two lots of rainbow trout (*Salmo gairdneri*) showed a variation of 1.6 gillrakers when exposed to incubation temperatures of 7 and 15°C, the higher number being associated with the higher temperature (Kwain 1975).

The large difference observed between spring and fall ciscoes from the rivière Kiamika basin in the number of gillrakers, coupled with the absence of overlap in the frequency distribution of gillrakers on the inferior limb, leads us to conclude that there are genotypic differences between these two forms. Natural selection acting through differences in the composition of the zooplankton fauna could cause such differences (Bailey and Smith 1981; Lindsey 1981), along with genetic drift, to which ciscoes are quite susceptible (Vuorinen et al. 1981). Natural selection and genetic drift can rapidly lead to higher fitness states (Suzuki et al. 1986).

Spring-spawning ciscoes from lac des Écorces also show lower counts than the fall spawners of adjacent lakes for the number of dorsal and anal fin rays and for the number of lateral line scales. Higher developmental temperatures could be responsible for these differences. For example, observed dif-

TABLE 3. Results of Scheffe's multiple comparison tests applied to the mean positions of samples of four stocks of rivière Kiamika basin ciscoes. Only the principal axes representing more than 5% of the total variance in the variance–covariance matrix of the transformed morphometric data were used.

	Conventional measures			Truss network measures		
Axis	% variance	$F_{(3,101)}$[a]	Scheffe[b]	% variance	$F_{(3,101)}$[a]	Scheffe[b]
I	35.9	22.3***	(S) (F, M) (F, K)	36.9	26.7***	(S, F, K) (F, M)
II	14.3	29.6***	(S, F, K) (M)	16.9	22.6***	(S, F) (F, K, M)
III	10.4	5.09**	(S, F, M) (K, F, M)	10.0	6.15***	(S, F, M) (F, K)
IV	8.0	1.63 NS	(S, F, K, M)	7.0	8.23***	(S, M) (F, K)
V	6.8	2.24 NS	(S, F, K, M)	5.7	0.74 NS	(S, F, M, K)

[a]NS, nonsignificant ($P > 0.05$); **$P < 0.01$; *** $P < 0.001$.

[b]Nonsignificantly different stocks ($P > 0.05$) are included in the same parentheses: S, lac des Écorces, spring; F, lac des Écorces, fall; M, lac Marquis; K, Petit lac Kiamika.

ferences in dorsal and anal fin rays between spring and fall ciscoes (1.2 and 1.1, respectively) are similar to those in *Salmo trutta* reported by Taning (1952): 1.5 for both fins for temperatures of 3–12°C. Lower counts of lateral line scales are also associated with higher developmental temperatures in *Coregonus* (Svärdson 1952) and more specifically in *C. trybomi* (Svärdson 1979).

This tendency was not observed in the paired fin ray counts. In fact, the average number of pelvic fin rays is higher in spring ciscoes than in the fall spawners of Petit lac Kiamika whereas the average number of pectoral fin rays is similar. Furthermore, five spring ciscoes showed a higher count (13) for pelvic fin rays than that reported (11 and 12) by Scott and Crossman (1974). It thus appears that the above conditions of development exist only very rarely in the distribution range of this species. Although Vladykov (1934) stated that individual variation in this character could not be assigned to environmental factors, Kwain (1975) showed that increases in temperature from 7 to 15°C and in light intensity during the incubation of rainbow trout eggs resulted in an average decrease of 0.85 ray in this fin. It therefore seems that the number of pelvic fin rays reacts differently to this environment, being comparable in this aspect with the pectoral fin (Barlow 1961; Kwain 1975).

The most notable morphometric differences between spring and fall spawners of both sexes are associated with the head and the caudal peduncle. Relatively smaller heads and eyes have also been observed in three of the four Swedish populations of spring-spawning ciscoes (Svärdson 1979). In these latter populations, this could be due to a higher growth rate in spring ciscoes (Svärdson 1979). This explanation remains speculative, as the growth of these fish was not studied. Hile (1937), however, has shown a negative relationship between growth rate and head size in ciscoes.

Clarke (1973) has shown that the morphology of lake cisco was strongly influenced by the morphometry and physicochemistry of the lake as well as by its climatic conditions related to its geographic location. These variables, however, do not explain differences observed between spring and fall ciscoes. For example, whereas an increase in the surface area of the lake and/or in total dissolved solids (TDS) should result in a larger head, jaw, and eye (Clarke 1973), we observe an inverse relationship between our two groups. Lac des Écorces is in fact larger than both Petit lac Kiamika and lac Marquis and has a higher TDS.

Martin (1949) has suggested some mechanisms of shape determination capable of explaining morphological differences between stocks of fishes. One can effectively describe the morphological development of a fish as being saltatory, each period (stanza) being coupled to an important physiological transformation and a net inflexion in the growth curve of an anatomical structure compared with body length. Four stanzas can thus be identified: embryonic stage, hatching, ossification, and sexual maturity (Martin 1949). In the population under study, early development of spring ciscoes is very rapid and the concerned stanza would probably correspond to ossification. In rainbow trout, this latter stanza appears at a length of about 30 mm (Martin 1949). Spring ciscoes reach this same length during the fall of their first year (Hénault 1986); subsequent low fall temperatures would cause these young fish to be subjected to a slower development resulting in a relatively smaller head, in comparison with fall ciscoes which arrive at this critical size at the beginning of summer. This could also explain why we observe an inverse relationship to the one mentioned by Martin (1949) between a small head size and high values for certain meristic counts. We cannot exclude, however, that the observed morphometric differences could also be due to size differences at sexual maturity, to the growth rate of adult individuals, or to genetic factors. Interestingly, the head length of *C. alpenae*, *C. hoyi*, and *C. kiyi* is little affected by the environment (Todd et al. 1981).

It is difficult to demonstrate the sympatric occurrence of a fall-spawning population of cisco in lac des Écorces, which could be suggested by the capture of some fall-spawning specimens. Although all of the generalized distances of Mahalanobis between stocks are significant, results from the principal components analysis invalidate such a conclusion. The fact that the canonical analysis does discriminate between the predefined categories does not mean that the variations are categorical (Thorpe 1983). Todd et al. (1981) have already encountered this contradiction in these two methods of analysis. As in the study by Todd et al. (1981), results of the principal components analysis seem to better reflect reality. In our study, fall ciscoes caught in lac des Écorces might not be native and could come for the most part from Petit lac Kiamika since, in a discriminant analysis involving spring ciscoes and the two upstream allopatric fall stocks, 80% of these specimens are classified in the Petit lac Kiamika stock and 20% in that of lac Marquis. The absence of differences in meristic counts, notably gillrakers, as well as the low number of captures of fall ciscoes in lac des Écorces (1.1% of the total number of captured ciscoes, with 2100 h of experimental fishing) tend to support the hypothesis of a downstream movement of fall spawners.

Differences in gillrakers and mouth parts associated with feeding, coupled with the electrophoretic similarity at 17 loci (Hénault 1986), are concordant with the competitive speciation model as applied to ciscoes by Smith and Todd (1984). This suggests that some competition for food resources could have existed in the past between these two ecotypes (Smith and Todd 1984). If we exclude the presence of a sympatric stock of fall spawners, we can then hypothesize that the two groups coexisted at the time of former glacial lac Gatineau and, if so, that the environmental conditions for spring spawning in *Coregonus* occur very rarely, since only two such populations are known despite many years of fisheries research.

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LETTERS AND COMMENTS/ LETTRES ET COMMENTAIRES

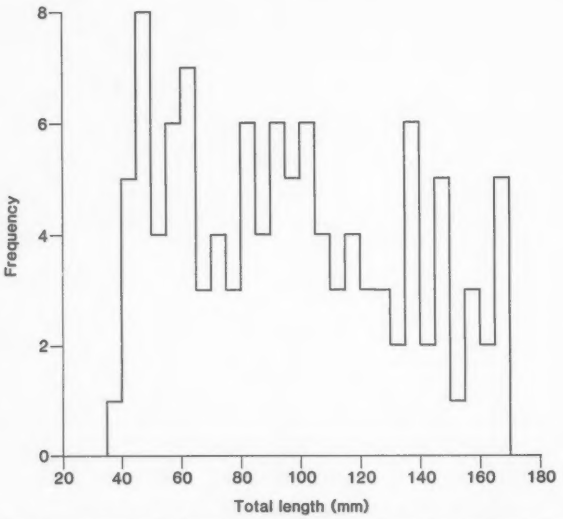

FIG. 1. Approximate length frequency distribution for the lamprey sample of Medland and Beamish (1987), constructed from the length data in the lower panel of their fig. 3.

Comment on Lamprey Length Frequency Distribution

In an age validation study, Medland and Beamish (1987, fig. 3) presented a length frequency distribution for 138 larvae of the mountain brook lamprey, *Ichthymyzon greelyi*, and compared it with an age–length plot for the same sample. In the figure caption it is stated that "The length frequency distribution...illustrates the difficulty sometimes encountered in identifying age groups." The authors appear to imply that length mode analysis of these data (using the methods of, e.g. MacDonald and Pitcher (1979) or Fournier and Breen (1983)) would produce erroneous growth estimates, since there are 12 clear length modes in the histogram but only five year classes in the sample.

This is misleading. The histogram cannot be what it is claimed to be. There are said to be 138 data and the mean frequency in the figure is at least 6, so there should be no more than 23 (= 138/6) length classes. In fact, 1-mm length classes appear to have been used. This would lead to approximately 135 length classes and an average frequency of about 1.

Figure 1 shows an approximate length frequency for the sample, derived by reading individual lengths from the lower panel of Medland and Beamish's figure. Perhaps because of overlapping points, I could locate only 111 data. The new figure does not present the many clear modes of Medland and Beamish's figure. A length mode analysis of these data may or may not produce accurate growth estimates. However, if it did not, it would not be because clear length modes did not correspond to the age–length data. In any case, the difference in male and female length frequencies evident from Medland and Beamish's figure would suggest that any modal analysis should be done separately by sex.

This comment is in no way intended to undermine the age validation presented by Medland and Beamish. Its aim is to prevent wrong inferences being drawn from their fig. 3 and to correct an error in that figure — R. I. C. C. Francis, *Fisheries Research Centre, P.O. Box 297, Wellington, New Zealand.*

(J9661)

References

FOURNIER, D. A., AND P. A. BREEN. 1983. Estimation of abalone mortality rates with growth analysis. Trans. Am. Fish. Soc. 112: 403–411.

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Response to Francis

While it is true that our figure caption stated "The length frequency distribution ... illustrates the difficulty sometimes encountered in identifying age groups," we in no way imply that there are 12 clear length modes in the length frequency distribution. The point being made is that discrimination of individual year classes is sometimes difficult and that a series of length frequency plots for animals from a given river but collected at intervals will suggest different numbers of age classes. The overlapping of the normal distributions around individual age classes in a length frequency analysis presents problems in assigning age to individual animals. It is in cases such as this that the information provided by statoliths is particularly valuable.

As for the length frequency distribution not being what it is claimed to be, I think the confusion could have been avoided if the figure caption had read "Length frequency distribution *adjusted by a moving average of seven (Ricker 1975)* ..." This omission is an error on our part. Lamprey were measured to the nearest millimetre, as stated in the materials and methods section. The data were then adjusted by a moving average of seven (Ricker 1975) and the adjusted length frequency plotted in fig. 3.

We would like to thank Dr. Francis for his constructive comments and we regret any confusion caused by the omission in the caption of fig. 3 — T. E. Medland, *Department of Zoology, University of Guelph, Guelph, Ont. N1G 2W1.*

(J9885)

References

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ANNOUNCEMENTS/AVIS

International Symposium on Coldwater Fish Culture

The International Symposium on Coldwater Fish Culture will be held 19–23 September 1989 in Beijing, China. For additional information, contact: Mr. Huang Kejia, The China Society of Fisheries, 31 Min Feng Lane, Xidan Beijing BJ100800, China. Telex: 22079 CNFC CN.

Conférence internationale sur la pisciculture en eau froide

Une conférence internationale sur la pisciculture en eau froide aura lieu le 19 au 23 septembre 1989 à Beijing en Chine. Pour plus de renseignements, communiquez avec : M. Huang Kejia, The China Society of Fisheries, 31 Min Feng Lane, Xidan Beijing BJ100800, China. Telex : 22079 CNFC CN.

Guide for Authors

Introduction

We welcome manuscripts reporting significant new information in fisheries and aquatic sciences. Manuscripts may concern cells, organisms, populations, ecosystems, or processes that affect aquatic production systems, and cover numerous disciplines including biology and ecology of marine and freshwater organisms, physiology, toxicology, genetics, limnology, oceanography, economics, disease, and management.

Manuscripts are selected for publication according to the extent and significance of new knowledge or ideas presented. Preference will be given to those whose experimental results emphasize causal mechanisms underlying observed phenomena rather than pure description and fact. Manuscripts should lead to identifiable conclusions or synthesis, which variously may amplify, modify, question, or redirect accumulated knowledge embodied in contemporary perceptions of a particular state of aquatic sciences. Their novelty must relate to more than the particular (a certain year, place, taxon, chemical compound) and their contribution to science must clearly be beyond the confirmatory state.

Manuscripts submitted should be as comprehensive as possible; research fragments are generally unacceptable. If a single paper cannot be produced, then closely related papers should be cross-referenced and submitted together. The paper should not be submitted or accepted for publication elsewhere.

The *Journal* considers the following types of contributions:

Articles: Studies of broad scope that are original contributions to science.

Reviews: Detailed critical appraisals of broad areas of investigation, particularly those that have developed rapidly in recent years.

Perspectives: Essays of opinion or hypothesis on aquatic science of concern to the professional and layman. References are desirable; informal style is welcome.

Comments: Opinion on topics recently discussed in the *Journal*; preferred maximum length, 500 words.

Manuscript Submission

Submit manuscripts in triplicate to the Editor, accompanied by a covering letter that (1) states the main points and significance of the work, (2) accepts the mandatory $44 page charge, (3) suggests potential referees, and (4) identifies other manuscripts containing the same or similar information. Original typescripts and figures (except halftones) are best retained by the author until requested.

Each manuscript is normally submitted to two referees for appraisal before final evaluation by an editor. However, the Editor will return unreviewed those manuscripts that do not fall within the *Journal's* editorial policy or scope.

Editorial Practices

The *Council of Biology Editors Style Manual* (5th ed. CBE, 9650 Rockville Pike, Bethesda, MD 20814) is used as the authority in matters of form. The standards for spelling are either *Webster's Third International Dictionary* or the *Oxford English Dictionary* and *Le Grand Larousse encyclopédique*. Authors are responsible for consistency in spelling. Titles of periodicals are abbreviated as in *Serial Sources for the BIOSIS Data Base* (BioSciences Information Service, Philadelphia, PA 19103). Authors are responsible for ensuring the accuracy and completeness of their reference list. The *Journal* follows the names and spelling recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (4th ed. 1980). Spec. Publ. No. 12, American Fisheries Society. All measurements must be metric, use SI symbols in most instances (exceptions noted in editorial in the *Journal*, Vol. 40, No. 12), and adhere to the *Canadian Metric Practice Guide* (CAN3-Z234.1-79 (Canadian Standards Association, 178 Rexdale Boulevard, Rexdale, Ont. M9W 1R3).

Series titles should be avoided. An abstract is required for all manuscripts and should state concisely, in up to 175 words, what was done, found, and concluded.

Proofs are sent to the author, who is responsible for correcting errors in typesetting. *Alterations to the content must be avoided.*

Fifty reprints are supplied free. Additional reprints may be purchased at the time of publication (an order form is sent with the proofs).

Preparation of Manuscripts

General Guidelines

Movement of manuscripts through review channels and the editorial office is greatly expedited if manuscripts are prepared in *Journal* style and format. When in doubt about specific details, consult a current issue of the *Journal*. General guidelines follow.

Type the manuscript on white paper, preferably 21.5 × 28 cm (8.5 × 11 in.), on only one side of the page. Leave all margins at least 2.5 cm wide.

Type only the title, authors' names and affiliations, and related footnotes on the first page. Number all pages beginning with the title page, including those for tables and captions for illustrations.

Double-space all parts of the manuscript, including tables, captions for tables and figures, footnotes, and the reference list. Underline only material that is to be printed in italics.

Do not use all capitals anywhere in the manuscript.

Use the name-and-year system for literature citations.

Checklist for Manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparisons? Are there inconsistencies between tables and figures and the text, or within the text? Should some of the data

be made available separately in a data or manuscript report or at a data repository?

If statistical analysis is included, is it subordinate to the research? When probability statements are made, are only the statistical tests cited and unnecessary statistical tables excluded?

Would any of the text be clearer if condensed? Are summary statements given at the beginning of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report? Does the *abstract* give the essentials of the new knowledge? Is the *introduction* largely limited to the scope, purpose, and rationale of the study? Is review of the literature limited to defining the problem? Are details of *materials and methods* limited to what scientists need to understand the design of the study and to judge the adequacy of the data? Are generalizations from the *results* supported by the data provided? Are findings distinguished from inferences? Is the *discussion* limited to interpretation and significance of the findings?

The most common technical problems in submitted manuscripts are listed below. Authors can hasten the processing of their papers if they pay attention to these points during manuscript preparation.

1) Insert bibliographic strip before the abstract (Authors, date, title, Can. J. Fish. Aquat. Sci.).

2) Limit abstract to one paragraph of about 175 words.

3) Double-space all elements of the manuscript, including references, table captions, and figure legends.

4) Avoid exclusive use of capitals everywhere in the manuscript, including headings, table captions, and figure legends.

5) Underline only Latin names of organisms and certain statistical and mathematical notation to indicate italics.

6) Use correct SI symbols for units or measure in figures, tables, and text. Place a zero before the decimal for numbers less than unity.

7) Avoid ambiguous forms such as g C/m²/d; use g $C \cdot m^{-2} \cdot d^{-1}$.

8) Identity test used to test statistical significance and give probability value. No reference is needed for common statistical tests.

9) In the text, generalize from tables and figures; avoid repeating all the details. Be sure each table and figure can stand on its own and is referred to in the text in numerical order. The captions should explain the purpose of the table or figure.

10) Include acknowledgments section at manuscript stage, not when galley proofs arrive.

11) Provide the person's initials and mailing address when referring to personal communications.

12) Delete unnecessary references that do not apply directly to the problem.

13) Check references carefully against test citations and vice versa to ensure exact correspondence. Provide an availability statement for less easily retrieved material, e.g. available from Department of Economics, Simon Fraser University, Burnaby, B.C. V5A 1S6.

14) Delete commas between name and date in citations such as (Smith 1900) and do not underline "et al."

15) Replace 10^n in table headings and figure axes with appropriate SI prefixes or use words (e.g. thousands).

16) Photocopies of figures, except halftones, are appropriate for review purposes. Send photographic prints of figures, or originals if they do not exceed 21.5 × 28 cm, on request for publication. Use the same type of lettering throughout if possible.

Parts of the Manuscript

Organization

Organize the manuscript on the basis of the purpose or scope of the study as stated in the introduction. Ensure that the title and headings are in harmony with the statement of purpose.

Before writing any of the manuscript, list tentative headings in as few ranks as possible. Rework them until they appear to allow logical development for the reader; usually, chronological order is not effective. The findings will be more readily appreciated if methods, findings, and discussion are given in separate sections.

Organize tables and figures to facilitate comparisons, grouping related data in as few tables and figures as feasible. As far as possible, make the tables and figures clear without reference to the text.

Begin sections and paragraphs with topic sentences containing generalizations that lead readily to the particulars. Giving a conclusion first and then supporting it not only improves readability but also facilitates assessment by other scientists. Failure to give the most newsworthy generalizations first is one of the most prominent shortcomings in presentation of manuscripts.

See that everything in each section is relevant to the heading, and everything in each paragraph to the topic (opening) sentence.

Before writing any paragraphs, try writing the topic sentences for all of them and arranging these in appropriate order.

Title

Limit the title to what is documented in the manuscript. It is the key to the article and should clearly and concisely reveal what appears in the paper itself. The title serves two functions: (1) it allows the reader to judge whether or not the article is of potential interest and (2) it should provide enough information to permit the reader to judge the scope and potential importance of the article. Words in the title should convey a maximum amount of information and identify the nature of the research, organism used, and where appropriate, the technical approach (e.g. X ray, chromatography, mathematical analysis). Titles should not begin with a numeral or introductory prepositions such as "On" or "Towards" or expressions such as "A contribution to . . ." or "Investigations on . . ." Good titles greatly assist scientists and librarians in using scientific literature and aid indexers preparing titles for keyword indexes.

Abstract

The abstract should succinctly mirror the content and emphasis of the paper in about 175 words. Like the title, the abstract enables readers to determine the paper's content and decide whether or not they need to read the entire article. Begin the abstract with the main conclusion from the study, and support it with the relevant findings. Limit details of methods to those needed in understanding what was done, and work them into statements of findings. Avoid using phrases such as ". . . is discussed" or ". . . was found"; be specific. As the abstract is often divorced from the main body of the paper by abstracting and indexing services and is the only part of a paper some read-

ers ever see, it is important that it accurately reflect the paper's contents.

Introduction

Limit the introduction largely to the scope, purpose, and rationale of the study. Restrict the literature review and other background information to that needed in defining the problem or setting the work in perspective. Try beginning with the purpose or scope of the work, defining the problem next, and adding guideposts to orient the reader. An introduction generally need not exceed 375–500 words.

Materials and Methods

Materials and Methods provides the framework for getting answers to the questions posed in the purpose of the work.

Limit the information on materials and methods to what is needed in judging whether the findings are valid. To facilitate assessment, give all the information in one section when possible. Refer to the literature concerning descriptions of equipment or techniques already published, detailing only adaptations. Often, it helps to begin statements on procedures with a phrase indicating the purpose, such as "To determine . . . we . . ." If the section is long, consider using subheadings corresponding to headings for the findings.

Results

Limit the results to answers to the questions posed in the purpose of the work, and digest them as comprehensively as possible. Give the findings as nearly as possible in the terms in which the observations or measurements were made and so avoid confusion between facts and inferences. State noteworthy findings to be noted in each table and figure, and avoid restating in the test what is clear from the titles. Material supplementary to the text can be archived in the report literature or a recognized data depository and referenced in the text.

Discussion

Limit the discussion to giving the main contributions of the study and interpreting particular findings, comparing them with those of other workers. Emphasis should be maintained on synthesis and interpretation and exposition of broadly applicable generalizations and principles. If these are exceptions or unsettled points, note them and show how the findings agree or contrast with previously published work. Limit speculation to what can be supported with reasonable evidence. End the discussion with a short summary of the significance of the work and conclusions drawn. If the discussion is brief and straightforward, it can be combined with the results section.

Acknowledgments

We strongly urge authors to limit acknowledgments to those who contributed substantially to scientific and technical aspects of the paper, gave financial support, or improved the quality of the presentation. Avoid acknowledging those whose contribution was clerical only.

References

References should be selected judiciously and be largely restricted to significant, published literature. References to unpublished data, manuscripts in preparation or submitted to other journals, progress reports, and unpublished papers given at annual meetings may not be cited in the reference list but may be noted in the text as unpublished data or personal communications (include mailing addresses). If consultants' reports or other documents of limited circulation must be cited, they should carry with them an availability statement explaining where the document can be obtained. Citations of literature in the text should be carefully checked against those in the reference list and vice versa to ensure exact correspondence. Nearly every manuscript submitted to the *Journal* contains errors in the references.

Tables

Tables are used to present repetitive data and should be as economical of space as possible.

Design tables to fit a 1-, 1½- or 2- column width of the *Journal*.

Type each on a separate page and number with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Table captions should be succinct and identify the purpose of the table sufficiently well to allow the table to stand on its own.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, are typed with the text, and seldom need horizontal lines.

Figures

Provide photographic reproductions or the original artwork (no larger than 21.5 × 28 cm) of each illustration. If submitting photographs, type the author's name and figure name on a strip of paper and fasten to the back of the print. Do not write on the illustration itself. Provide two photocopies of line illustrations and two sets of any photographs for review purposes.

Design figures to fit a 1-, 1½-, or 2-column width of the *Journal* and ensure that all lettering, numerals, and symbols are legible, neat, and at least 1.5 mm high when reduced. Figures should not be mounted, stapled, or clipped. When possible, figures should be grouped and be done with the same or similar type of lettering.

Type legends in succession on a separate page. Add bar scales to maps, photomicrographs, equipment diagrams, etc.

Where each figure is first referred to, type "Fig. — near here."

Computer-generated figures should be avoided if possible, as they usually reproduce poorly. Dot-matrix printers cannot produce sufficiently dark, solid lines to ensure legibility. Figures drafted in black india ink are always preferable. If sheets of printed characters are used, be sure they adhere completely.

Draft rather than type axis labels for graphs.

Color plates are very expensive to produce and should only be used where the color being presented is essential to the understanding of the text. Extra costs for the reproduction of color plates are usually charged to the author. Submit only the most outstanding photographs for color reproduction. We pre-

fer to use color photographs, but will accept drawings, color negatives, slides, or film positives.

Mathematical Expressions, Equations, and Formulae

Leave three spaces above and below lines that have much material in superscripts, and above and below equations and formulae. In the various expressions, place each component in its correct position.

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter *l* and the number *one*, and between the capital *O* and the numeral *zero*. Make other explanatory notes similarly.

Appendix

Abbreviations

Abbreviate terms denoting units of weight and measurement in the text only when they are preceded by numerals.

becquerel	Bq
calorie	cal
centimetre	cm
centimetre, square	cm^2
centimetre, cubic	cm^3
centimetre per gram per second	$cm \cdot g^{-1} \cdot s^{-1}$
coulomb	C
day	d
decimetre	dm
degree Celsius	°C
degrees of freedom	df
gram	g
hectare	ha
hertz	Hz
hour	h
joule	J
kilometre	km
litre	L
lumen	lm
lux	lx
metre	m
metre, square	m^2
metre, cubic	m^3
micrometre	µm
milligram	mg
millilitre	mL
millimetre	mm
millimetre, square	mm^2
minute	min
molar mass	M
mole	mol
mole per litre	mol/L, M
month	mo
Pascal	Pa
second	s
standard deviation	SD
standard error	SE
tonne	t
volt	V
volume	vol
watt	W
week	wk
year	yr

Dates

Dates may be written in the sequence day-month-year without internal punctuation (On 9 October 1983 the . . .), or the alternative sequence month-day-year may be employed with the year set off by commas before and after it (On October 9, 1983, the . . .).

Reference Citations in Text

Name-and-year system

The *Journal* uses the name-and-year system of citation; that is, the surname of the author(s) and the year of publication are inserted in the text at an appropriate point: "Brown (1983) compared . . ." or ". . . were compared (Brown 1983)." If the reference has more than two authors, include only the surname of the first author followed by "et al." (not italicized): "Brown et al. (1983) compared . . ." or ". . . were compared (Brown et al. 1983)."

Personal communications

Personal communications are not listed in the reference list. Using parentheses in the text, state the name and mailing address of the communicator followed by "pers. commun."

Unpublished data

If the unpublished book or article has been accepted for publication, list it in the reference list section followed by the notation "(In press)." Only those manuscripts that are in galley or page proof or for which there is an acceptance letter can be considered in press. If the article is submitted but not yet accepted, state the name and address of the author of the unpublished material followed by the notation "unpubl. data" in the text and do not include it in the reference list.

Reference Lists

The following bibliographic citations illustrate the punctuation, style, and abbreviations (according to *Serial Sources for the BIOSIS Data Base*) for references.

Journal article

PETERMAN, R. M. 1982. Model of salmon age structure and its use in preseason forecasting and studies of marine survival. Can. J. Fish. Aquat. Sci. 39: 1444–1452.

Entire issue of journal

GORDON, D. C. JR., AND A. S. HOURSTON [ED.] 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. Can. J. Fish. Aquat. Sci. 40(Suppl. 1): 1–365.

Book in a series

SCOTT, W. B., AND E. J. CROSSMAN. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. 184: 966 p.

Book not in a series

LEBLOND, P. H., AND L. A. MYSAK. 1978. Waves in the ocean. Elsevier, New York, NY. 602 p.

Part of book

HEALEY, M. C. 1980. The ecology of juvenile salmon in Georgia Strait, British Columbia. p. 203–229. *In* W. J. Neil and D. C. Himsworth [ed.] Salmonid ecosystems of the North Pacific. Oregon State University Press, Corvallis, OR.

Corporate author

AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. 1975. Standard

methods for the examination of water and wastewater. 14th ed. Washington, DC. 1193 p.

Theses

KUTTY, M. N. 1966. Some studies on the respiratory quotient in goldfish and rainbow trout. Ph.D. thesis, Univ. Toronto, Toronto, Ont. 102 p. Natl. Libr. Can., Can. Theses Microfilm No. 646.

Reports

SMITH, J. E. 1981. Catch and effort statistics of the Canadian groundfish fishery on the Pacific coast in 1980. Can. Tech. Rep. Fish. Aquat. Sci. 1032: 90 p.

Translation

KOIKE, A., AND M. OGURA. 1977. Selectivity of meshes and entrances of shrimp traps and crab traps. J. Tokyo Univ. Fish. 64: 1–11. (Transl. from Japanese by Can. Transl. Fish. Aquat. Sci. 4950, 1983)

Time

A colon should be used as the separator between hour and minute and between minute and second. The symbols "h," "min," and "s" are not used, since they are the symbols for hour, minute, and second in the sense of duration or the length of time. Thus "12 h 30 min" expresses a measured time of twelve hours and thirty minutes duration whereas 12:30 refers to the time of day.

Word List

The spelling of the following words is frequently inconsistent in submitted manuscripts. We prefer that authors adhere to the *Journal's* house style for these commonly used terms:

age-class (n.)
age-group (n.)
aquaculture (n.)
Arctic char (n.)
brackish water (n.)
brackish-water (adj.)
chi-square (n., adj.)
cold water (n.)
cold-water (adj.)
deep sea (n.)
deep-sea (adj.)
deep water (n.)
deepwater (adj.)
freshwater (n., adj.)
fresh water (n.)
groundwater (n., adj.)
hard water (n.)
hardwater (adj.)
headwater (n., adj.)
lake water (n., adj.)
meltwater (n., adj.)
open water (n.)
open-water (adj.)
percent (n.)
salt water (n.)
saltwater (adj.)
sea-run (adj.)
seawater (n., adj.)
shallow water (n.)
shallow-water (adj.)
short term (n.)
size-class (n.)
snowmelt (n.)
soft water (n.)
softwater (adj.)
tidewater (n., adj.)
t-test (n., adj.)
warm water (n.)
warmwater (adj.)
year-class (n.)
young-of-the-year (n., adj.)

Guide des auteurs

Introduction

Les manuscrits comportant une information nouvelle dans le domaine des sciences halieutiques et aquatiques sont les bienvenus. Ils peuvent se rapporter aux cellules, aux organismes, aux populations, aux écosystèmes ou aux processus qui influencent les systèmes de production aquatiques, et couvrir de nombreuses disciplines, y compris biologie et écologie des organismes marins et dulçaquicoles, physiologie, toxicologie, génétique, limnologie, océanographie, économique, maladies et gestion.

Les critères de sélection des manuscrits dépendent de l'étendue et de l'importance des connaissances ou idées nouvelles qu'ils contiennent. On donnera la préférence à ceux dont les résultats expérimentaux font ressortir les mécanismes sousjacents aux phénomènes observés plutôt qu'une simple description ou énoncé de fait. Ils doivent aboutir à des conclusions ou synthèses précises qui, de diverses façons, peuvent accroître, modifier, remettre en question ou réorienter le bagage actuel des connaissances et perceptions dans une discipline donnée des sciences aquatiques. L'originalité doit dépasser le caractère particulier (une année, un endroit, un taxon ou un composé chimique donné) et tenir à une épuration ou à une reformulation des connaissances actuelles en faisant plus que corroborer des faits.

Les manuscrits soumis devraient être aussi complets que possible; des fragments de recherche sont généralement inacceptables. S'il est impossible de produire un seul article, on devra soumettre ensemble des articles étroitement liés entre eux, avec références croisées. L'article ne doit pas avoir été soumis ou accepté pour publication ailleurs.

Le *Journal* considère les types de contributions suivants :

Articles : études d'une portée générale, constituant des contributions inédites à la science.

Critiques : appréciations critiques détaillées de vastes champs de recherche, particulièrement dans les domaines ayant connu de rapides progrès au cours des dernières années.

Perspectives : essais portant sur une opinion ou une hypothèse relative aux sciences aquatiques qui intéressent professionnels et profanes. Il est préférable de donner des références et d'écrire, si possible, dans une style simple.

Commentaires : opinions sur des sujets récemment exposés dans le *Journal*, et, de préférence, ne dépassant pas 500 mots.

Soumission des manuscrits

Les manuscrits doivent être soumis au rédacteur en triplicata, et accompagnés d'une lettre mentionnant : 1) les principaux points et l'importance du travail, 2) l'acceptation des frais de publication obligatoires de 44 $ par page, 3) des suggestions quant aux arbitres éventuels et 4) une mention d'autres manuscrits contenant une information identique ou qui se rapproche de celle contenue dans le manuscrit soumis. L'auteur devra, de préférence, conserver les originaux du texte dactylographié et des figures (sauf les simili) jusqu'à ce qu'on les lui demande.

Un manuscrit est normalement soumis à deux arbitres pour évaluation, avant l'évaluation finale par un rédacteur. Cependant, les manuscrits qui ne répondent pas à la politique éditoriale ou à la portée du *Journal* sont retournés à l'auteur sans avoir été soumis à l'arbitrage.

Dispositions générales

Le *Council of Biology Editors Style Manual* (5e éd., CBE, 9650 Rockville Pike, Bethesda, MD 20814) sert de guide quant à la forme de la présentation. Le *Webster's Third International Dictionary* ou l'*Oxford English Dictionary*, et le *Grand Larousse encyclopédique* font autorité pour l'orthographe des mots. L'uniformité de cette dernière est la responsabilité des auteurs. Les titres de périodiques sont abrégés suivant les *Serial Sources for the BIOSIS Data Base* (BioSciences Information Service, Philadelphia, PA 19103). C'est la responsabilité des auteurs de s'assurer que leurs références sont complètes et exactes. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (4e éd. 1980), Spec. Publ. No. 12, American Fisheries Society. Toutes les mesures doivent être conformes au système métrique et, dans la plupart des cas, être exprimées en unités SI (sauf exceptions mentionnées dans un éditorial du *Journal*, vol. 40, no 12), et se conformer au *Guide canadien de familiarisation au système métrique* CAN3-Z234.1-79 (l'Association canadienne de normalisation, 178, boul. Rexdale, Rexdale (Ont.) M9W 1R3).

On devra éviter les titres en séries. Un résumé doit accompagner chaque texte et exposer brièvement, en 175 mots ou moins, ce qui a été fait, ce qui a été découvert et les conclusions auxquelles on est arrivé.

L'auteur doit revoir les épreuves qu'on lui remet et en corriger les erreurs typographiques. *Il doit éviter de modifier le contenu du texte.*

Cinquante tirés à part sont fournis gratuitement. Des tirés à part supplémentaires peuvent être achetés au moment de la publication (un bon de commande à cet affet accompagne les épreuves).

Préparation des manuscrits

Directives générales

La marche des manuscrits à travers les diverses étapes de l'arbitrage et de la révision sera grandement facilitée si les articles sont préparés dans le style et le format du *Journal*. En cas de doute sur des points particuliers, veuillez consulter une livraison récente du *Journal*. On trouvera ci-dessous des directives générales.

Dactylographiez le manuscrit sur papier blanc, mesurant de préférence 21,5 sur 28 cm (8,5 sur 11 pouces), sur un côté de la page. Laissez toujours une marge d'au moins 2,5 cm.

Sur la première page, dactylographiez le titre, les noms et affiliations des auteurs et les renvois qui s'y rapportent. Numérotez toutes les pages, à commencer par la page-titre, de

même que celles contenant les tableaux et les légendes des illustrations.

Tout le manuscrit, y compris tableaux, légendes des tableaux et des figures, renvois et liste de références, doit être dactylographié à double interligne. Ne soulignez que les mots qui devront être imprimés en italique.

En aucun endroit du manuscrit n'écrivez entièrement en majuscules.

Pour les références citées dans le texte, utilisez le système nom et année.

Liste de contrôle pour manuscrits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les rédacteurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes aux buts de l'étude ?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons ? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou à l'intérieur du texte ? Est-ce que certaines données devraient être présentées séparément dans un rapport manuscrit ou dans un dépôt de données ?

Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la recherche ? Dans des énoncés de probabilité, seuls les tests statistiques sont-ils cités, et les tableaux statistiques inutiles sont-ils exclus ?

Le texte y gagnerait-il en clarté s'il plus condensé, en tout ou en partie ? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents au sujet traité ? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction ?

Le *titre* est-il représentatif du contenu du rapport ? Le *résumé donne-t-il l'essentiel des connaissances nouvelles ? L'introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude ? La revue de la littérature se limite-t-elle à la définition du problème ? La section *matériel et méthodes* contient-elle le minimum de détail qui permettront aux autres scientifiques de saisir les grandes lignes de l'étude et de juger de la valeur des données ? Les généralisations sur les *résultats* sont-elles adéquates ? Permettent-elles de distinguer entre constatations et déductions ? La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes ?

Sont énumérés ci-dessous des façons d'éviter les problèmes techniques les plus communs dans la soumission des manuscrits. En accordant une attention particulière à ces points lors de la préparation de leurs manuscrits, les auteurs peuvent accélérer le traitement des articles.

1) Insérez la bande bibliographique avant le résumé (auteurs, date, titre, Can. J. Fish. Aquat. Sci.)

2) Limitez le résumé à un paragraphe d'environ 175 mots.

3) Dactylographiez à double interligne tous les éléments du manuscrit, y compris les références et les légendes des tableaux et des figures.

4) Évitez partout l'emploi exclusif de majuscules, y compris dans les en-têtes et les légendes des tableaux et des figures.

5) Ne soulignez que les noms latins d'organismes et certaines notations statistiques et mathématiques, qui devront être imprimés en italique.

6) Dans les figures, les tableaux et le texte, exprimez les unités de mesure par leurs symboles SI propres. Pour les nombres inférieurs à l'unité, placez un zéro avant la virgule décimale.

7) Évitez les formes ambiguës, telles que g C/m^2/d ; utilisez plutôt g C·m^{-2}·d^{-1}.

8) Identifiez le test qui a servi à évaluer la signification statistique, donnant également la valeur de probabilité. S'il s'agit de tests communs, il n'est pas nécessaire d'en donner la référence.

9) Dans le texte, généralisez à partir des tableaux et des figures ; évitez une répétition de tous les détails. Assurez-vous que tous les tableaux et figures sont indépendants et que, dans le texte, on y réfère dans l'ordre numérique. Les légendes doivent indiquer le but du tableau ou de la figure.

10) Préparez la section des remerciements au stade du manuscrit et non quand vous parviennent les épreuves.

11) En référant à des communications personnelles, donnez les initiales et l'adresse postale de la personne en cause.

12) Éliminez toutes références inutiles qui ne s'appliquent pas directement au problème.

13) Vérifiez soigneusement les références en regard des citations dans le texte et vice versa, afin de vous assurer qu'il y a correspondance. Dans le cas de matériel difficilement accessible, indiquez l'endroit où le trouver, p. ex., disponible auprès du Department d'économique, Université Simon Fraser, Burnaby (C.-B.) V5A 1S6.

14) Éliminez les virgules entre le nom et la date dans les citations telles que (Smith 1900) et soulignez *et al.*

15) Dans les en-têtes de tableaux et les axes des figures, remplacez 10^n par les préfixes SI appropriés ou utilisez des mots en clair (p. ex. milliers).

16) Aux fins de la révision, les photocopies des figures, sauf les similis, sont appropriées. Sur demande, lorsqu'il s'agira de publier l'article, envoyez des photographies des figures, ou encore les originaux, s'ils ne dépassent pas 21,5 sur 38 cm. Utilisez autant que possible un lettrage uniforme.

Parties du manuscrit

Organisation

Organisez le manuscrit par rapport au but ou à la portée de l'étude, tels qu'énoncés dans l'introduction. Il est particulièrement important que le titre et les sous-titres soient en harmonie avec les objectifs.

Avant de commencer la rédaction du manuscrit, dressez une liste provisoire des sous-titres ou en-têtes, avec le moins de divisions possible. Retouchez-les jusqu'à ce qu'ils semblent assurer, pour le lecteur, une marche logique ; ordinairement, l'ordre chronologique ne sera pas satisfaisant. Il sera plus facile d'evaluer les résultats si vous décrivez méthodes, résultats et discussion dans des sections séparées.

Organisez les tableaux et les figures de façon à faciliter les comparaisons, groupant les données apparentées en aussi peu de tableaux et de figures que possible. Assurez-vous que ces derniers sont aussi clairs que possible sans référence au texte.

Commencez les sections et les paragraphes par des énoncés généraux qui aboutissent naturellement à des énoncés particuliers. Si vous donnez d'abord une conclusion que vous appuyez ensuite, non seulement votre travail sera plus facile à lire, mais aussi les autres scientifiques pourront plus aisément en évaluer les résultats. Dans la rédaction des manuscrits, le défaut le plus commun est d'omettre de donner, au début, les généralités les plus dignes de mention.

Voyez à ce que le matériel de chaque section soit pertinent à l'en-tête et que le matériel de chaque paragraphe se rapporte à la première phrase introduisant le sujet.

Avant de rédiger les paragraphes, composez la phrase-thème de chaque paragraphe et disposez ces phrases dans l'ordre approprié.

Titre

Limitez le titre à ce que le travail veut démontrer. C'est la clé de l'article, et il devrait montrer clairement et de façon concise ce qui est contenu dans le travail lui-même. Le titre a deux fonctions : 1) il permet au lecteur de juger si, oui ou non, l'article peut avoir un intérêt et 2) il devrait fournir suffisamment d'information pour permettre au lecteur de juger de la portée et de l'importance possible de l'article. Les mots du titre devraient fournir un maximum d'information et identifier la nature de la recherche, de l'organisme utilisé et, au besoin, la méthode d'approche technique (p. ex. rayons X, chromatographie, analyse mathématique). Les titres ne devraient pas commencer par un chiffre ou par des prépositions d'introduction telles que «Sur» ou «Vers», ou des expressions telles que «Contribution à . . .» ou «Recherches sur . . .». De bons titres facilitent le classement des publications et leur utilisation par les scientifiques et les bibliothécaires.

Résumé

Le résumé doit refléter succintement le contenu et les points principaux de l'article en 175 mots environ. Comme le titre, le résumé permet aux lecteurs de prendre connaissance du contenu de l'article et de décider si, oui ou non, il leur faut lire ce dernier en entier. Indiquez au début du résumé la principale conclusion de l'étude et appuyez-la par des découvertes pertinentes. Ne donnez que les détails des méthodes nécessaires à la compréhension de ce qui a été fait et insérez-les dans l'énoncé des découvertes. Évitez l'emploi de phrases telles que «. . . on y analyse» ou «. . . a été trouvé» ; soyez spécifique. Comme le résumé est souvent séparé du corps principal de l'article par les services d'analyse et de classement, et que c'est la seule partie d'un travail que certains lecteurs auront l'occasion de voir, il est important que le résumé reflète précisément le contenu du travail.

Introduction

Limitez l'introduction à la portée, au but et à la raison de l'étude. Ne mentionnez que les travaux publiés et autres informations nécessaires à la définition du problème et à la perspective du travail. Commencez avec le but ou la portée du travail, définissez ensuite le problème et ajoutez ici et là des jalons qui aideront à orienter le lecteur. En général, une introduction n'a pas besoin de dépasser 375–500 mots.

Matériel et méthodes

Cette section renferme ce dont on a besoin pour trouver réponse aux questions soulevées dans les objectifs du travail.

Limitez les renseignements à ce qui est nécessaire pour juger de la validité des découvertes. Cette évaluation sera facilitée si vous présentez, autant que possible, toute l'information dans une section. S'il s'agit d'outillage ou de techniques déjà publiés, référez à ces publications et ne décrivez en détail que les adaptations. Il y a souvent avantage à commencer un énoncé des méthodes par une phrase qui en indique le but, comme : «Afin de déterminer . . . nous . . .» Si la section est longue, songez à utiliser des sous-titres correspondant à ceux que vous utilisez dans la section des résultats.

Résultats

Limitez les résultats aux réponses apportées aux questions en rapport avec le but de l'étude ; que ces réponses soient aussi complètes que possibles, tout en étant concises. Exprimez ce que vous avez trouvé, autant que possible dans les mêmes termes que ceux dans lesquels les observations ou les mesures ont été faites, évitant ainsi de confondre faits et spéculations. En dégageant les généralités des tableaux et des figures, référez-y et évitez de répéter dans le texte ce que les légendes devraient exprimer clairement. Le matériel complémentaire au texte peut être classé dans les séries des rapports ou dans un dépôt de données reconnu, et on y référera dans le texte.

Discussion

Limitez la discussion à l'énoncé de la principale contribution de l'étude et à l'interprétation de découvertes particulières, les comparant avec celles d'autres auteurs. On continuera à mettre l'accent sur la synthèse et l'interprétation, ainsi que sur l'exposition de généralisations et de principes largement applicables. S'il s'agit d'exceptions ou de points douteux, notez-les et faites ressortir la manière dont vos résultats corroborent ou contredisent les travaux déjà publiés. Limitez la spéculation à ce que vous pouvez appuyer sur des preuves raisonnables. Terminez cette section par un court résumé de l'importance du travail et des conclusions auxquelles il a conduit. Si la discussion est brève et précise, elle peut être combinée avec les résultats en une seule et même section.

Remerciements

Nous recommandons fortement aux auteurs de limiter les remerciements aux personnes qui ont contribué d'une manière substantielle aux aspects scientifiques et techniques de l'article, fourni une aide financière ou amélioré la qualité de la présentation. Évitez de mentionner les personnes qui n'ont fourni qu'un travail de soutien.

Références

On choisira les références avec soin, les limitant aux publications importantes. On ne peut citer dans cette section les références à des données inédites, manuscrits en préparation ou soumis à d'autres périodiques, états des travaux et communications inédites présentées à des réunions annuelles. On peut toutefois les mentionner dans le texte à titre de données inédites ou de communications personnelles (incluant les adresses). Si l'on doit citer des rapports d'experts-conseils ou d'autres documents dont la distribution est limitée, on devra préciser l'endroit où l'on peut se les procurer. On doit s'assurer que les références mentionnées dans le texte correspondent à celles qui sont contenues dans la liste de références et vice versa. Presque tous les manuscrits soumis au *Journal* contiennent des erreurs dans cette section.

Tableaux

Les tableaux sont utilisés pour présenter des données qui se répètent et devraient occuper le moins d'espace possible.

Agencez les tableaux de façon qu'ils s'adaptent soit à 1, 1½- ou 2 colonnes du *Journal*.

Dactylographiez chaque tableau sur une page séparée et numérotez-les en chiffres arabes.

Tracez des lignes horizontales de part et d'autre des en-têtes et au-dessous des colonnes, mais rarement ailleurs. N'utilisez

jamais de lignes verticales. Laissez plutôt des espaces supplementaires.

Chaque tableau doit avoir une légende instructive, complète et indépendante.

Les renvois des tableaux doivent être indiqués par des petites lettres suscrites dactylographiées au bas du tableau.

Placez les tableaux après la liste des références.

Quand on réfère à un tableau pour la première fois, on indique dans la marge du manuscrit «Tableau — près d'ici».

Notez que les *tableaux dans le texte* ne sont pas numérotés et sont dactylographiés dans le corps du texte. Ils ont rarement besoin de lignes horizontales.

Figures

Fournissez des photographies ou les dessins originaux (pourvu qu'ils ne dépassent pas 21,5 sur 28 cm) de chaque illustration. Dactylographiez le nom de l'auteur et le numéro de la figure sur une lisière de papier que vous collerez au dos. N'écrivez pas sur l'illustration même. Transmettez deux photocopies des dessins au trait et deux séries de photos qui serviront au moment de la révision.

Les dessins devront occuper une largeur de 1, 1½- ou 2 colonnes du *Journal*. Assurez-vous que tous les chiffres, lettres et symboles sont lisibles, clairs et mesurent au moins 1,5 mm de hauteur une fois réduits. Les figures ne devraient pas être montées, brochées ou fixées à l'aide de trombones. Autant que possible, elle devraient être groupées et préparées avec le même type de lettrage.

Dactylographiez les légendes, dans l'ordre, sur des feuilles séparées. Dans le cas de cartes, photomicrographies, diagrammes d'instruments, etc., ajoutez une échelle.

À l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge «Fig. — près d'ici».

On devra autant que possible éviter les illustrations préparées par ordinateur, qui ne se reproduisent pas très bien. Les imprimantes par points ne peuvent produire de lignes solides suffisamment foncées pour être lisibles. On obtiendra toujours de meilleurs résultats en utilisant de l'encre de Chine noire. Si vous utilisez du lettrage instantané, veillez à ce qu'il adhère parfaitement.

Il vaut mieux dessiner que dactylographier les inscriptions relatives aux axes.

La production de planches en couleurs est très dispendieuse et on ne devrait utiliser celles-ci que lorsque les couleurs sont essentielles à la compréhension du texte. Les coûts supplémentaires de reproduction de planches en couleurs sont ordinairement débités à l'auteur. Pour les reproductions en couleurs, ne soumettez que des photos d'excellente qualité. Nous préférons utiliser des photographies en couleur, mais acceptons dessins, négatifs, diapositives ou positifs de films en couleurs.

Expressions, équations et formules mathématiques

Laissez trois espaces au-dessus et au-dessous des lignes portant des chiffres ou des lettres suscrits et souscrits, ou encore au-dessus et au-dessous des équations et des formules. Placez chaque composante des différentes expressions dans la position qui lui est propre.

Indiquez clairement tous les symboles. Épelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre *l* et le chiffre *un*, et entre la majuscule *O* et le chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Appendice

Abréviations

N'abrégez les termes désignant les unités de poids et mesures dans la texte que lorsqu'ils sont précédés d'un nombre.

an	an
année	a
becquerel	Bq
calorie	cal
centimètre	cm
centimètre carré	cm^2
centimètre cube	cm^3
centimètre par gamme par seconde	$cm \cdot g^{-1} \cdot s^{-1}$
coulomb	C
décimètre	cm
degré Celsius	°C
degrés de liberté	dl
écart type	ET
élecronvolt	eV
erreur type	ErT
farad	F
gramme	g
hectare	ha
hertz	Hz
heure	h
joule	J
jour	d
kilomètre	km
litre	L
lumen	lm
lux	lx
mètre	m
mètre caré	m^2
mètre cube	m^3
micromètre	µm
milligramme	mg
millilitre	mL
millimètre	mm
millimètre carré	mm^2
minute	min
mois	mo
masse molaire	M
mole	mol
moles par litre	mol/L, M
pascal	Pa
seconde	s
semaine	sem
tonne	t
volt	V
volume	vol
watt	W

Dates

On écrira les dates dans l'ordre jour-mois-année, p.ex. 9 octobre 1983, sans virgule entre le mois et l'année.

Citations de références dans le texte

Système de nom et année

Le *Journal* utilise le système de nom et année, c.-à-d. que le nom de l'auteur ou des auteurs et l'année de publication sont insérés dans le texte à un endroit approprié : «Brown (1983)

compare . . .» ou «. . . on a comparé (Brown 1983)». Si la référence comporte plus de deux auteurs, inscrivez le nom du premier auteur seulement, suivi de «et al.» : «Brown et al. (1983) comparent . . .» ou «. . . on a comparé (Brown et al. 1983).»

Communications personnelles

Les communications personnelles ne doivent pas être incluses dans la liste des références. Utilisez plutôt des parenthèses dans le texte, entre lesquelles vous indiquerez le nom et l'adresse de la personne en question, suivis de «comm. pers».

Données inédites

Si le livre ou l'article inédits ont été acceptés pour publication, insérez-les dans la liste des références, avec la mention «(Sous presse)». On ne peut considérer comme étant sous presse que les manuscrits au stade d'épreuves ou ceux couverts par lettre d'acceptation. Si l'article a été soumis mais n'a pas encore été accepté, ne l'incluez pas dans la liste de références, mais indiquez le nom et l'adresse de l'auteur du matériel inédit, dans le texte lui-même, avec la mention «données inédites».

Liste de références

La liste qui suit donne des exemples de ponctuation, de style et d'abréviations utilisés dans les références bibliographiques (d'aprés les *Serial Sources for the BIOSIS Data Base*).

Article du journal

ARCHAMBAULT, D. ET E. BOURGET. 1983. Importance du régime de dénudation sur la structure et la succession des communautés intertidales de substrat rocheux en milieu subarctique. Can. J. Fish. Aquat. Sci. 40: 1278–1292.

Numéro complet du journal

GORDON, D. C. JR. ET A. S. HOURSTON ÉD.] 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. Can. J. Fish Aquat. Sci. 40: (Suppl. 1): 1–365.

Livre dans une série

HILTON, J. W. ET S. J. SLINGER. 1981. Nutrition et alimentation de la truite arc-en-ciel. Publ. spéc. can. sci. halieut. aquat. 55 : 17 p.

Livre ne faisant pas partie d'une série

FORRESTER, W. D. 1983. Manuel canadien des marées. Ministère des Pêches et des Océans. Service hydrographique du Canada, Ottawa (Ont.) 148 p.

Partie d'un livre

AUBERT, M., J. AUBERT, S. DANIEL ET N. DESIROTTE. 1982. État actuel de la pollution bactérienne au large des côtes françaises, p. 36–40. *Dans* M. Ruivo [éd.] La pollution des mers et les ressources biologiques. Fishing News (Books) Ltd., Londres.

Corporation-auteur

AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION ET WATER POLLUTION CONTROL FEDERATION. 1975. Standard methods for the examination of water and wastewater. 14e éd. Washington, DC. 1193 p.

Thèse

DELISLE, C. 1969. Écologie, croissance et comportement de l'éperlan du Lac Heney, Comté de Gatineau ainsi que la répartition en eau douce du Québec. Thèse doctorale, Univ. Ottawa, Ottawa (Ont.). 161 p.

Rapports

DUTIL, D. J., M. FORTIN ET Y. VIGNEAULT. 1982. L'importance des zones littorales pour les ressources halieutiques. Mémoire remis au Conseil consultatif de l'environnement du Québec. Rapp. manus. can. halieut. aquat 1653 : 29 p.

Traduction

KOIKE, A. ET M. OGURA. 1977. Selectivity of meshes and entrances of shrimp traps and crab traps. J. Tokyo Univ. Fish. 64: 1–11. (Traduit du japonais par Can. Transl. Fish Aquat. Sci. 4950, 1983)

Temps

On devra séparer par deux points (:) heures et minutes, et minutes et secondes. Les symboles «h,» «min» et «s» ne seront pas utilisés car ils représentent heure, minute et seconde dans le sens de durée ou intervalle de temps de douze heures et trente minutes, alors que 12:30 se rapporte à l'heure du jour.

Liste de mots

Cette section, dans la version anglaise du Guide, contient une série de mots dont l'orthographe pose certains problèmes d'uniformité, comme, par exemple, «cold water» (n.) «cold-water» (adj.); «hard water» (n.), «hardwater» (adj.), etc. L'auteur préparant une texte en anglais est prié de consulter la version anglaise du Guide.

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